



**Eur päisches
Patentamt**

**European
Patent Office**

**Office européen
des brevets**

Bescheinigung

Certificate

Attestation

Die angehefteten Unterla-
gen stimmen mit der
ursprünglich eingereichten
Fassung der auf dem näch-
sten Blatt bezeichneten
europäischen Patentanmel-
dung überein.

The attached documents
are exact copies of the
European patent application
described on the following
page, as originally filed.

Les documents fixés à
cette attestation sont
conformes à la version
initialement déposée de
la demande de brevet
européen spécifiée à la
page suivante.

Patentanmeldung Nr. Patent application No. Demande de brevet n°

02018686.2

Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
p.o.

R C van Dijk



Anmeldung Nr:
Application no.: 02018686.2
Demande no:

Anmeldetag:
Date of filing: 21.08.02
Date de dépôt:

Anmelder/Applicant(s)/Demandeur(s):

Boehringer Ingelheim International GmbH
Binger Strasse 173
55216 Ingelheim am Rhein
ALLEMAGNE

Bezeichnung der Erfindung/Title of the invention/Titre de l'invention:
(Falls die Bezeichnung der Erfindung nicht angegeben ist, siehe Beschreibung.
If no title is shown please refer to the description.
Si aucun titre n'est indiqué se referer à la description.)

Compositions and methods for treating cancer using cytotoxic CD44 antibody
immunoconjugates and chemotherapeutic agents

In Anspruch genommene Priorität(en) / Priority(ies) claimed /Priorité(s)
revendiquée(s)

Staat/Tag/Aktenzeichen/State/Date/File no./Pays/Date/Numéro de dépôt:

Internationale Patentklassifikation/International Patent Classification/
Classification internationale des brevets:

A61K47/48

Am Anmeldetag benannte Vertragstaaten/Contracting states designated at date of
filing/Etats contractants désignées lors du dépôt:

AT BE BG CH CY CZ DE DK EE ES FI FR GB GR IE IT LI LU MC NL PT SE SK TR

21. Aug. 2002

Compositions and methods for treating cancer using cytotoxic CD44 antibody immunoconjugates and chemotherapeutic agents

The invention relates to the combined use of conjugates of antibodies with cytotoxic
5 compounds and chemotherapeutic agents in cancer therapy, pharmaceutical compositions comprising such compounds and/or chemotherapeutic agents, and methods of cancer treatment.

There have been numerous attempts to improve the efficacy of antineoplastic drugs by
10 conjugating such drugs to antibodies against tumor-associated antigens in order to elevate local concentration of the drug by targeted delivery to the tumor. Many of these approaches have met limited success, and several reasons have been discussed in the literature to explain the failure. For anticancer drugs acting stoichiometrically, like e.g. doxorubicin or methotrexate, relatively high intracellular concentrations are necessary to exert the required
15 cytotoxicity. These concentrations are thought to be difficult to achieve with many antibody-drug conjugates because of (a) insufficient potency of many common anticancer drugs, (b) low cell surface concentration of antigen targets, (c) inefficient internalization of antigen-antibody complexes into the target cell, and (d) inefficient release of free drug from the conjugate inside the target cell (Chari et al., 1992).

20

Two of the aforementioned drawbacks, namely (a) and (d), have been addressed by the work of Chari and coworkers (Chari et al., 1992; Liu et al., 1996; U.S. Patent No. 5,208,020).

They have developed antibody conjugates wherein the antibody is linked to a maytansinoid via a disulfide linkage. Maytansines belong to the class of Ansa macrolide antibiotics,
25 which derive from *Nocardia* sp.. The maytansine ansamitocin P-3, produced by bacterial fermentation, is used as a precursor molecule to manufacture maytansinoid DM1.

Maytansine and derivatives act as anti-mitotic agents (inhibitors of tubulin polymerization), similar as vincristine, but with markedly higher potency than vincristine or other established chemotherapeutic agents (DM1 is toxic to cells *in vitro* at $\sim 10^{-10}$ M
30 concentration). In contrast to the high cytotoxicity of free maytansinoid, the antibody conjugate has a toxicity which is several orders of magnitude lower on antigen-negative cells compared to antigen-positive cells. The linkage by disulfide bonding has the

advantage that these bonds are readily cleaved inside the target cells by intracellular glutathione, releasing highly toxic free drug. This approach has been applied to antibodies against tumor-associated antigens, for example the C242-DM1 conjugate (Liu et al., 1996; Lambert et al., 1998), and HuN901-DM1 (Chari et al., 2000). However, the application of these conjugates is restricted due to the limited expression of the respective target antigens. For example, the antigen recognized by N901 (CD56, N-CAM) is predominantly expressed by tumors of neuroendocrine origin, the expression of the C242 antigen (CanAg) is mostly limited to tumors derived from the GI tract.

There is, therefore, still the need to improve this approach by finding suitable tumor-associated antibodies with favorable antigen expression pattern, high and specific cell surface antigen concentration within the target tissue, and efficient internalization process transporting the antigen-complexed antibody conjugate into the cells.

CD44 is a protein which is expressed in several different isoforms on the surface of a wide variety of cell types. The smallest isoform, standard CD44 (CD44s), which is expressed by a variety of different cells, is thought to mediate cell attachment to extracellular matrix components and may transmit a co-stimulus in lymphocyte and monocyte activation. In contrast, expression of splice variants of CD44 which contain the domain v6 (CD44v6) in the extracellular region, is restricted to a subset of epithelia. The physiological role of CD44v6 is not yet fully understood.

CD44v6, as well as other variant exons (CD44v3, CD44v5, CD44v7/v8, CD44v10) has been shown to be a tumor-associated antigen with a favorable expression pattern in human tumors and normal tissues (Heider et al., 1995; Heider et al., 1996; Dall et al., 1996; Beham-Schmid et al., 1998; Tempfer et al., 1998; Wagner et al., 1998) and has been subject to antibody-based diagnostic and therapeutic approaches, in particular radioimmunotherapy (RIT) of tumors (Verel et al., 2002; Stromer et al., 2000, WO 95/33771, WO 97/21104).

However, a prerequisite for efficient killing of tumor cells by antibody maytansinoid conjugates is sufficient internalization of the target antigen. Only few data on the internalization of CD44 are available. Bazil et Horejsi reported that downregulation of

CD44 on leukocytes upon stimulation with PMA is caused by shedding of the antigen rather than by internalization (Bazil et Horejsi, 1992). Shedding of CD44 is also supported by several reports on soluble CD44 in the serum of tumor patients and normal individuals (Sliutz et al., 1995; Guo et al., 1994; Martin et al., 1997). In a recent paper by Aguiar et al. the amount of internalized CD44 on matrix-intact chondrocytes was determined to be approximately 6% in 4 hours (Aguiar et al., 1999). Similar low levels of internalized CD44v6 on tumor cells were found in experiments performed by BIA. Taken together, these data suggest that CD44 receptors are more likely subject to shedding than to internalization, and thus CD44 specific antibodies are not to be regarded as suitable candidates for the maytansinoid conjugate approach. This has been supported by in vitro cell proliferation assays wherein Ab_{CD44v6}-DM1 showed only slightly elevated cytotoxicity against antigen-presenting cells as compared to cells lacking the antigen.

It now has been found that CD44 specific antibodies conjugated to highly cytotoxic drugs through a linker which is cleaved under intracellular conditions are very efficient tumor therapeutics in vivo. Thus, such compounds may be advantageously used in cancer therapy.

There is still the need for further improvements. For the antibody maytansinoid conjugates huN901-DM1 and C242-DM1, the combination of these conjugates with the taxanes paclitaxel or docetaxel has been suggested (WO 01/24763).

It has now been unexpectedly found that the combination of a conjugate consisting of a CD44 specific antibody and a cytotoxic agent with a further chemotherapeutic agent shows synergistic effects.

25

In particular, the present invention relates to the use of a compound of formula

A(LB)_n (Formula (I))

wherein

- A** is an antibody molecule which is specific for CD44;
- 30 **L** is a linker moiety;
- B** is a compound which is toxic to cells; and
- n** is a decimal number with $n = 1$ to 10

for the preparation of a pharmaceutical composition for the treatment of cancer, wherein said compound is used or is for use in combination with a further chemotherapeutic agent. In a further aspect, the present invention relates to a method of treatment of cancer, wherein an effective amount of a compound of Formula (I), as defined herein, is administered to a patient in need thereof in combination with a further chemotherapeutic agent.

The antibody molecule A has a binding specificity for CD44, preferably variant CD44, most preferably CD44v6.

10

The term „antibody molecule“ shall encompass complete immunoglobulins as they are produced by lymphocytes and for example present in blood sera, monoclonal antibodies secreted by hybridoma cell lines, polypeptides produced by recombinant expression in host cells which have the binding specificity of immunoglobulins or monoclonal antibodies, and molecules which have been derived from such immunoglobulins, monoclonal antibodies, or polypeptides by further processing while retaining their binding specificity. In particular, the term „antibody molecule“ includes complete immunoglobulins comprising two heavy chains and two light chains, fragments of such immunoglobulins like Fab, Fab', or F(ab)₂ fragments (Kreitman *et al.*, 1993), recombinantly produced polypeptides like chimeric, humanised or fully human antibodies (Breitling *et al.*, 1999; Shin *et al.*, 1989; Güssow *et al.*, 1991, Winter *et al.*, 1994, EP 0 239 400; EP 0 519 596; WO 90/07861 EP 0 368 684; EP 0 438 310; WO 92/07075; WO 92/22653; EP 0 680 040; EP 0 451 216), single chain antibodies (scFv, Johnson *et al.*, 1991), multimeric antibodies (Kortt *et al.*, 2001) like diabodies, triabodies, or tetrabodies, and the like. Today, antibodies may also be produced without immunising a laboratory animal, e.g. by phage display methods (Aujame *et al.*, 1997; US 5,885,793; US 5,969,108; US 6,300,064; US 6,248,516, US 6,291,158). Fully human antibodies may be produced using transgenic mice carrying functional human Ig genes (EP 0 438 474 ; EP 0 463 151; EP 0 546 073). From the aforementioned literature references, the expert knows how to produce these types of antibody molecules, employing state of the art methods like automated peptide and nucleic acid synthesis, laboratory animal immunisation, hybridoma technologies, polymerase chain reaction (PCR), vector and expression technologies, host cell culture, and protein purification methods. In the following, the terms „antibody“ and „antibody molecule“ are used interchangeably.

30

„Specific for CD44“ shall mean that the antibody molecule has specific binding affinity for an epitope present in CD44. In a preferred embodiment, the antibody molecule of the invention has a binding specificity for the amino acid sequence coded by variant exon v6 of the human CD44 gene. The sequence of variant exon v6 as well as of the other variant exons is known in the art (Screaton *et al.*, 1992; Tölg *et al.*, 1993; Hofmann *et al.*, 1991). A preferred antibody molecule of the invention specifically binds to peptides or polypeptides having or containing the amino acid sequence SEQ ID NO: 1 of the accompanying sequence listing, or an allelic variant of said sequence. Preferably, said antibody molecule has binding specificity for an epitope within said sequence. More preferably, the antibody molecule specifically binds to a peptide having the amino acid sequence SEQ ID NO: 2, even more preferably having the amino acid sequence SEQ ID NO: 3. Such antibody molecules may be easily produced with methods known in the art (WO 95/33771, WO 97/21104), e.g. by immunising laboratory animals with chemically synthesised peptides having the aforementioned sequences, e.g. bound to a hapten, or immunising with a recombinantly produced fusion protein including said sequences, and proceeding according to methods known in the art (Harlow 1988; Catty 1988; Koopman *et al.*, 1993; Heider *et al.*, 1993).

Preferably, an antibody molecule to be used for the present invention is the murine monoclonal antibody with the designation VFF-18 which is produced by a hybridoma cell line which has been deposited on 07 June 1994 under the accession number DSM ACC2174 with the DSM-Deutsche Sammlung für Mikroorganismen und Zellkulturen GmbH, Mascheroder Weg 1b, D-38124 Braunschweig, Deutschland/Germany. Also preferred are Fab, Fab', or F(ab)₂ fragments of said monoclonal antibody VFF-18. In another preferred embodiment, the antibody molecule is a humanised recombinant antibody, wherein the complementarity determining regions (CDR's) of VFF-18 have been grafted into the respective genes of human immunoglobulin heavy and light chains.

"Complementarity determining regions" of a monoclonal antibody are understood to be those amino acid sequences involved in specific antigen binding according to Kabat *et al.*, 1991, in connection with Chothia and Lesk, 1987.

In another preferred embodiment, appropriate framework residues of such a CDR-grafted antibody are reverted to murine residues to improve binding affinity. From methods pertinent to the art, the experts knows how to obtain the CDR's of VFF-18, starting with the aforementioned hybridoma with the accession number DSM ACC2174, to choose and obtain appropriate human immunoglobulin genes, to graft the CDR's into these genes, to modify selected framework residues, to express the CDR-grafted antibody in appropriate host cells, e.g. Chinese hamster ovary (CHO) cells, and to test the resulting recombinant antibodies for binding affinity and specificity (see e.g. literature references above). In another preferred embodiment of the invention, the antibody molecule is a recombinant antibody having the CDR's of the antibody VFF-18. Preferably, such a recombinant antibody is a humanised antibody and is a complete immunoglobulin consisting of two complete light and two complete heavy chains. In another preferred embodiment of the invention, the antibody molecule is a recombinant antibody having the same idiotype as the antibody VFF-18. In another preferred embodiment of the invention, the antibody molecule is a recombinant antibody binding to the same epitope as the antibody VFF-18.

In a particular preferred embodiment, the antibody molecule A is an antibody comprising light chains having the amino acid sequence SEQ ID NO: 4, and heavy chains having the amino acid sequence SEQ ID NO: 6. This antibody is called BIWA 4. It is a humanised version of antibody VFF-18 mentioned above, having the complementary determining regions of the murine monoclonal antibody VFF-18 in a completely human framework, and human constant regions. It is therefore an antibody of very low immunogenicity in man, which is a favorable trait. However, as it has no murine framework residues to optimise antigen binding, it has a significantly lower antigen binding affinity as its parent antibody VFF-18, and therefore would not have been regarded as a good candidate for a therapeutic drug. Unexpectedly, it has been found that BIWA 4, despite its poor binding affinity, has a very favorable biodistribution and tumor uptake in vivo, making it superior to other humanised versions of VFF-18 with higher binding affinity. In a further preferred embodiment, the antibody molecule A is an antibody comprising light chains having the amino acid sequence SEQ ID NO: 8, and heavy chains having amino acid sequence SEQ ID NO: 6. This antibody is called BIWA 8 and has higher binding affinity than BIWA 4.

These antibodies may be produced as follows. Nucleic acid molecules coding for the light chain and the heavy chain may be synthesised chemically and enzymatically by standard methods. First, suitable oligonucleotides can be synthesized with methods known in the art (e.g. Gait MJ, 1984), which can be used to produce a synthetic gene. Methods to generate synthetic genes from oligonucleotides are known in the art (e.g. Stemmer et al. 1995; Ye et al. 1992; Hayden et Mandecki 1988; Frank et al. 1987). Preferably, the nucleic acid molecules encoding the light and heavy chains of BIWA 4 have the nucleotide sequences of SEQ ID NO: 5 and SEQ ID NO: 7, respectively. These sequences include sequences coding for leader peptides which are cleaved by the host cell (SEQ ID NO: 5: the first 60 nucleotides; SEQ ID NO: 7: the first 57 nucleotides). In a further embodiment, the nucleic acid molecules encoding the light and heavy chains of an antibody molecule according to the invention have the nucleotide sequences of SEQ ID NO: 9 and SEQ ID NO: 7, respectively. These nucleic acid molecules encoding the antibody heavy and light chains then may be cloned into an expression vector (either both chains in one vector molecule, or each chain into a separate vector molecule), which then is introduced into a host cell. Expression vectors suitable for immunoglobulin expression in prokaryotic or eukaryotic host cells and methods of introduction of vectors into host cells are well-known in the art. In general, the immunoglobulin gene therein is in functional connection with a suitable promoter, like for example a human cytomegalovirus (CMV) promoter, hamster ubiquitin promoter (WO 97/15664), or a simian virus SV40 promoter located upstream of the Ig gene. For termination of transcription, a suitable termination/polyadenylation site like that of the bovine growth hormone or SV40 may be employed. Furthermore, an enhancer sequence may be included, like the CMV or SV40 enhancer. Usually, the expression vector furthermore contains selection marker genes like the dihydrofolate reductase (DHFR), glutamine synthetase, adenosine deaminase, adenylate deaminase genes, or the neomycin, bleomycin, or puromycin resistance genes. A variety of expression vectors are commercially available from companies such as Stratagene, La Jolla, CA; Invitrogen, Carlsbad, CA; Promega, Madison, WI or BD Biosciences Clontech, Palo Alto, CA. For example, expression vectors pAD-CMV1 (NCBI GenBank Accession No. A32111) or pAD-CMV19 (NCBI GenBank Accession No. A32110) may be used for expression. The host cell preferably is a mammalian host cell, e.g. a COS, CHO, or BHK cell, more preferably a Chinese hamster ovary (CHO) cell, e.g. a CHO-DUKX (Urlaub and Chasin, 1980), CHO-DG44 (Urlaub et al., 1983), or CHO-K1 (ATCC CCL-61) cell. The host cell

then is cultured in a suitable culture medium under conditions where the antibody is produced, and the antibody is then isolated from the culture according to standard procedures. Procedures for production of antibodies from recombinant DNA in host cells and respective expression vectors are well-known in the art (see e.g. WO 94/11523, WO 5 97/9351, EP 0 481 790, EP 0 669 986).

In order to link the antibody molecule **A** to the compound **B** which is toxic to cells, a linking moiety **L** is used. In the most simple case, the linking moiety **L** is a chemical bond, preferably a covalent bond which is cleaved under intracellular conditions. In one 10 embodiment of the invention, the bond is between a sulfur atom present in the antibody molecule, e.g. in the side chain of a cystein residue, and another sulfur atom present in the toxic compound. In another embodiment, the linking moiety **L** consists of one or more atoms or chemical groups. Suitable linking groups are well known in the art and include disulfide groups, thioether groups, acid labile groups, photolabile groups, peptidase labile 15 groups and esterase labile groups. Preferred are disulfide groups and thioether groups.

Conjugates of the antibody molecules of the invention and toxic compound can be formed using any techniques presently known or later developed. The toxic compound can be modified to yield a free amino group and then linked to the antibody molecule via an acid- 20 labile linker, or a photolabile linker. The toxic compound can be condensed with a peptide and subsequently linked to an antibody molecule to produce a peptidase-labile linker. The toxic compound can be treated to yield a primary hydroxyl group, which can be succinylated and linked to an antibody molecule to produce a conjugate that can be cleaved by intracellular esterases to liberate free drug. Most preferably, the toxic compound is 25 treated to create a free or protected thiol group, and then one or many disulfide or thiol-containing toxic compounds are covalently linked to the antibody molecule via disulfide bond(s).

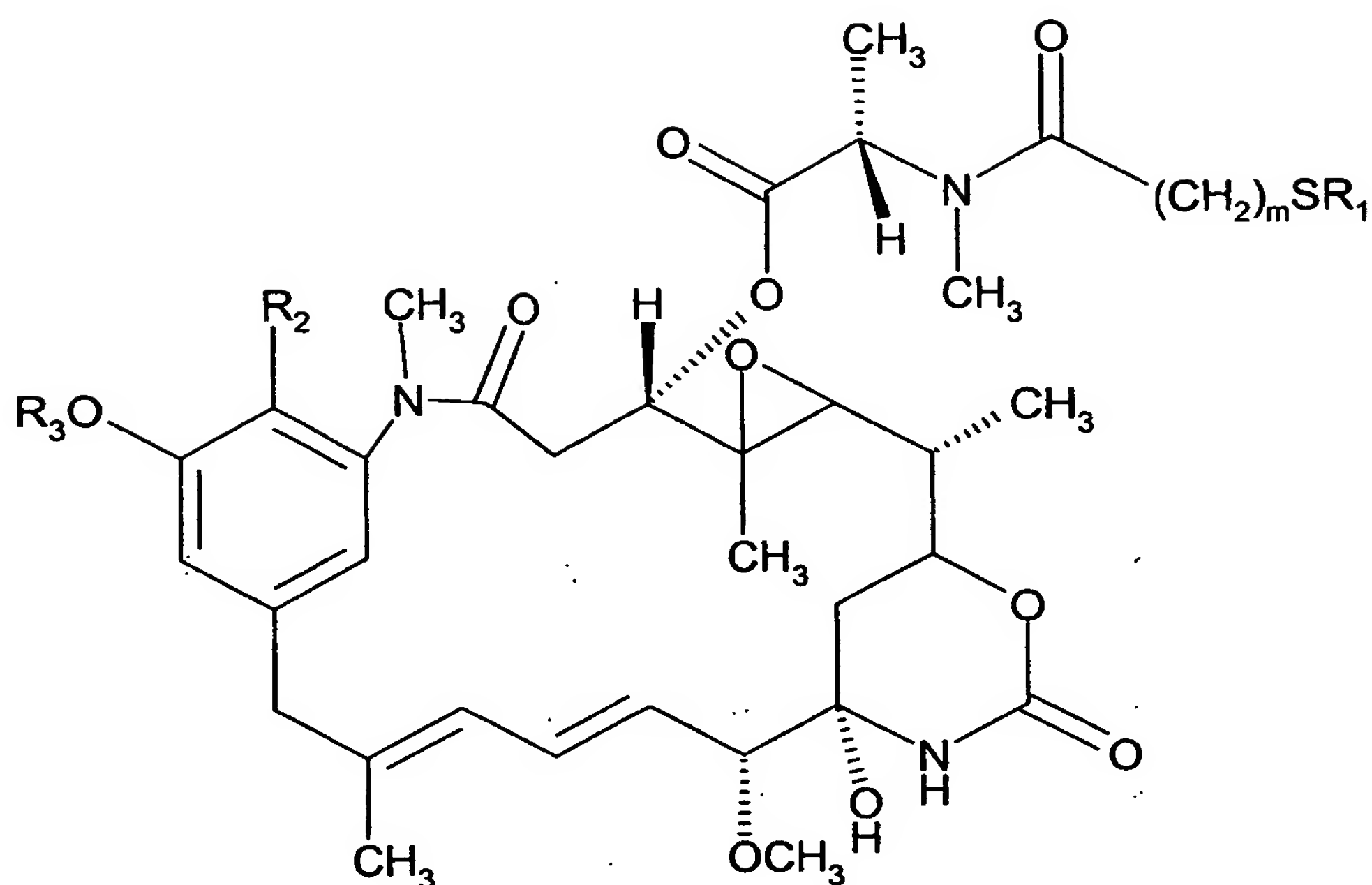
For example, antibody molecules can be modified with crosslinking reagents such as N- 30 succinimidyl 3-(2-pyridyldithio)propionate (SPDP), 4-succinimidyl-oxycarbonyl- α -methyl- α -(2-pyridyldithio)-toluene (SMPT), N-succinimidyl-3-(2-pyridyldithio)-butyrate (SDPB), N-succinimidyl-4-(2-pyridyldithio)pentanoate (SPP), N-succinimidyl-5-(2-pyridyldithio)pentanoate, 2-iminothiolane, or acetylsuccinic anhydride by known methods.

See, Carlsson et al, 1978; Blattler et al 1985; Lambert et al, 1983; Klotz et al, 1962; Liu et al, 1979; Blakey and Thorpe, 1988; Worrell et al, 1986. In a preferred embodiment, the linker moiety is a 5-thiopentanoate or 4-thiopentanoate derived from SPP. The antibody molecule containing free or protected thiol groups thus derived is then reacted with a disulfide- or thiol-containing toxic compound to produce conjugates. The conjugates can be purified by HPLC or by gel filtration.

„Toxic“ is a compound that inhibits or prevents function of cells and/or causes cell destruction. Toxic compounds used for coupling may act either cytostatic or cytotoxic and lead to cell cycle arrest or cell death. These compounds may act at different stages during the cell cycle, e.g. by interference with nucleic acid synthesis, inactivation of nucleic acids, or by binding to tubulin.

In a preferred embodiment, the compound **B** present in $A(LB)_n$ which is toxic to cells is a maytansinoid, i.e. a derivative of maytansine (CAS 35846538). In a preferred embodiment, it is a C-3 ester of maytansinol. Maytansinoids suitable for conjugating to antibodies for use in cancer therapy, including preparation of said maytansinoids and their linkage to antibody molecules, have been described by Chari and Coworkers (Chari et al., 1992; Liu et al., 1996; U.S. Patent No. 5,208,020). These maytansinoids may be used for the present invention. In a preferred embodiment, the toxic compound is $N^{2'}$ -deacetyl- $N^{2'}$ -(3-mercapto-1-oxopropyl)-Maytansine (CAS Number 139504-50-0), also referred to as DM1. Preferably, said maytansinoid is a maytansinol derivative linked to the antibody molecule via a disulfide bridge at the C-3 position of maytansinol. In a particularly preferred embodiment, the antibody/maytansinoid conjugate may be prepared from a maytansinoid of formula

Formula (II)



wherein

R_1 represents H or SR_4 , wherein R_4 represents methyl, ethyl, linear alkyl, branched alkyl, cyclic alkyl, simple or substituted aryl, or heterocyclic;

R_2 represents Cl or H;

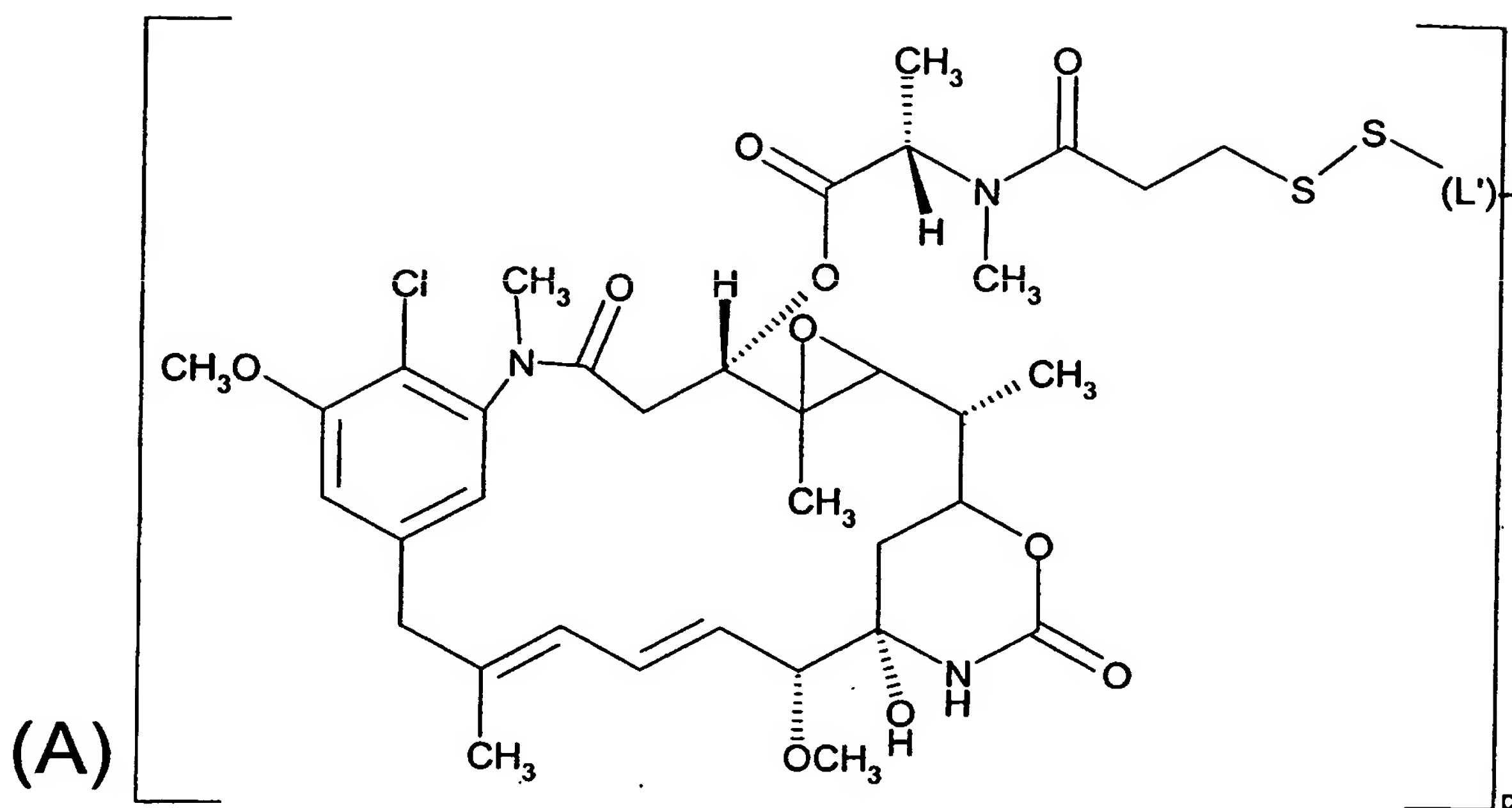
R_3 represents H or CH_3 ; and

m represents 1, 2, or 3.

Preferably, R_1 is H, CH_3 , or SCH_3 , R_2 is Cl, R_3 is CH_3 , and $m = 2$.

The compound with $R_1 = H$, $R_2 = Cl$, $R_3 = CH_3$, and $m = 2$ is designated DM1 in the literature.

In a preferred embodiment, the compound of the invention has the formula



(Formula III)

wherein

- 5 A is an antibody molecule which is specific for CD44, preferably specific for the variant exon v6, preferably specific for the amino acid sequence SEQ ID NO: 3;

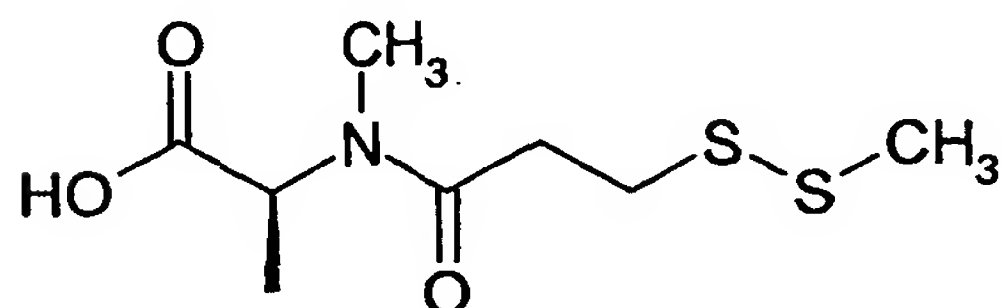
(L') is an optional linker moiety

p is a decimal number with $p = 1$ to 10

- 10 Preferably, p is 3 to 4, more preferably about 3.5.

Methods for preparing such maytansinoids are known in the art (see in particular US 5,208,020, Example 1). Conveniently, in a first step the maytansinoid C-3 ester ansamitocin P3 may be produced by bacterial fermentation (US 4,356,265; US 4,450,234; 15 WO 01/77360) of microorganisms belonging to the genus *Nocardia* or *Actinosynnema*, e.g. ATCC 31565, ATCC 31281. Ansamitocin P3 may be extracted from the culture using organic solvents like ethyl acetate or toluene, and further purified by adsorption chromatography using e.g. silica gel. It may then be reduced to maytansinol using LiAlH₄ (US 4,360,462) or, as suggested more recently (WO 02/16368), LiAl(OMe)₃H or other 20 LiAl or NaAl hydrides. The maytansinol may then be esterified at the C-3 position with N-methyl-L-alanine or N-methyl-L-cysteine derivatives to yield a disulfide-containing maytansinoid (US 5,208,020; US 5,416,064; US 6,333,410), for example using

dicyclohexylcarbodiimide(DCC) and catalytic amounts of zinc chloride (US 4,137,230; US 4,260,609). In a preferred embodiment, the maytansinol is esterified with the compound N-methyl-N-(3-methyldithiopropionyl)-L-alanine of formula



5

to yield the maytansinoid of Formula (II) with with $R_1 = SR_4$, $R_4 = CH_3$, $R_2 = Cl$, $R_3 = CH_3$, and $m = 2$. Manufacture of N-methyl-L-alanine or N-methyl-L-cysteine derivatives is disclosed for example in US 5,208,020 and WO 02/22554.

- 10 The free thiol group may then be released by cleavage of the disulfide bond with dithiothreitol (DTT), to yield e.g. DM1.

Upon intracellular cleavage, the free toxic compound is released. The free drug released from the compound $A(LB)_n$ may have the formula $B-X$, wherein X is an atom or a
 15 chemical group, depending on the nature of the cleaving reaction. Preferably, X is a hydrogen atom, as for example when the linker moiety is just a covalent bond between two sulfur atoms, or a hydroxyl group. The cleavage site may also be within the linker moiety if the linker moiety is a chemical group, generating free drug of formula $B-L''-X$ upon
 20 cleavage, wherein X is an atom or a chemical group, depending on the nature of the cleaving reaction. Preferably, X is a hydrogen atom or a hydroxyl group. The free drug released intracellularly may also contain parts (amino acid or peptidic residues) of the antibody molecule, if the linker is stable, but the antibody molecule is degraded.

In a preferred embodiment, the compound of Formula (I) is less toxic than the toxic
 25 compound B , $B-X$ or $B-L''-X$ released upon intracellular cleavage. Methods of testing cytotoxicity in vitro are known in the art (Goldmacher et al., 1985; Goldmacher et al., 1986; see also US 5,208,020, Example 2). Preferably, the compound (I) is 10 times or more, more preferably 100 times or more, or even 1000 times or more less toxic than the free drug released upon cleavage.

Preferably, antibody molecule/maytansinoid conjugates are those that are joined via a disulfide bond, as discussed above, that are capable of delivering maytansinoid molecules. Such cell binding conjugates are prepared by known methods such as modifying monoclonal antibodies with succinimidyl pyridyl-dithiopropionate (SPDP) or pentanoate (SPP; N-succinimidyl-4-(2-pyridyldithio)pentanoate, or N-succinimidyl-5-(2-pyridyldithio)pentanoate) (Carlsson et al, 1978). The resulting thiopyridyl group is then displaced by treatment with thiol-containing maytansinoids to produce disulfide linked conjugates. Alternatively, in the case of the arylthiomaytansinoids, the formation of the antibody conjugate is effected by direct displacement of the aryl-thiol of the maytansinoid by sulfhydryl groups previously introduced into antibody molecules. Conjugates containing 1 to 10 maytansinoid drugs linked via a disulfide bridge are readily prepared by either method. In this context, it is understood that the decimal number n in the formula $A(LB)_n$ is an average number as not all conjugate molecules of a given preparation may have the identical integer of LB residues attached to the antibody molecule.

More specifically, a solution of the dithiopyridyl modified antibody at a concentration of 1 mg/ml in 0.1 M potassium phosphate buffer, at pH 7.0 containing 1 mM EDTA is treated with the thiol-containing maytansinoid (1.25 molar equivalent/dithiopyridyl group). The release of pyridine-2-thione from the modified antibody is monitored spectrophotometrically at 343nm and is complete in about 30 min. The antibody-maytansinoid conjugate is purified and freed of unreacted drug and other low molecular weight material by gel filtration through a column of Sephadex G-25. The number of maytansinoids bound per antibody molecule can be determined by measuring the ratio of the absorbance at 252 nm and 280 nm. An average of 1-10 maytansinoid molecules/antibody molecule can be linked via disulfide bonds by this method.

In a preferred aspect, the present invention relates to a conjugate of a CD44v6 specific antibody molecule and a maytansinoid. Herein, „CD44v6 specific“ shall mean that the antibody has specific binding affinity to an epitope which is present in a peptide having the amino acid sequence encoded by variant exon v6 of CD44, preferably human CD44. A preferred antibody molecule of the invention specifically binds to peptides or polypeptides having or containing the amino acid sequence SEQ ID NO: 1 of the accompanying sequence listing, or an allelic variant of said sequence. Preferably, said antibody molecule

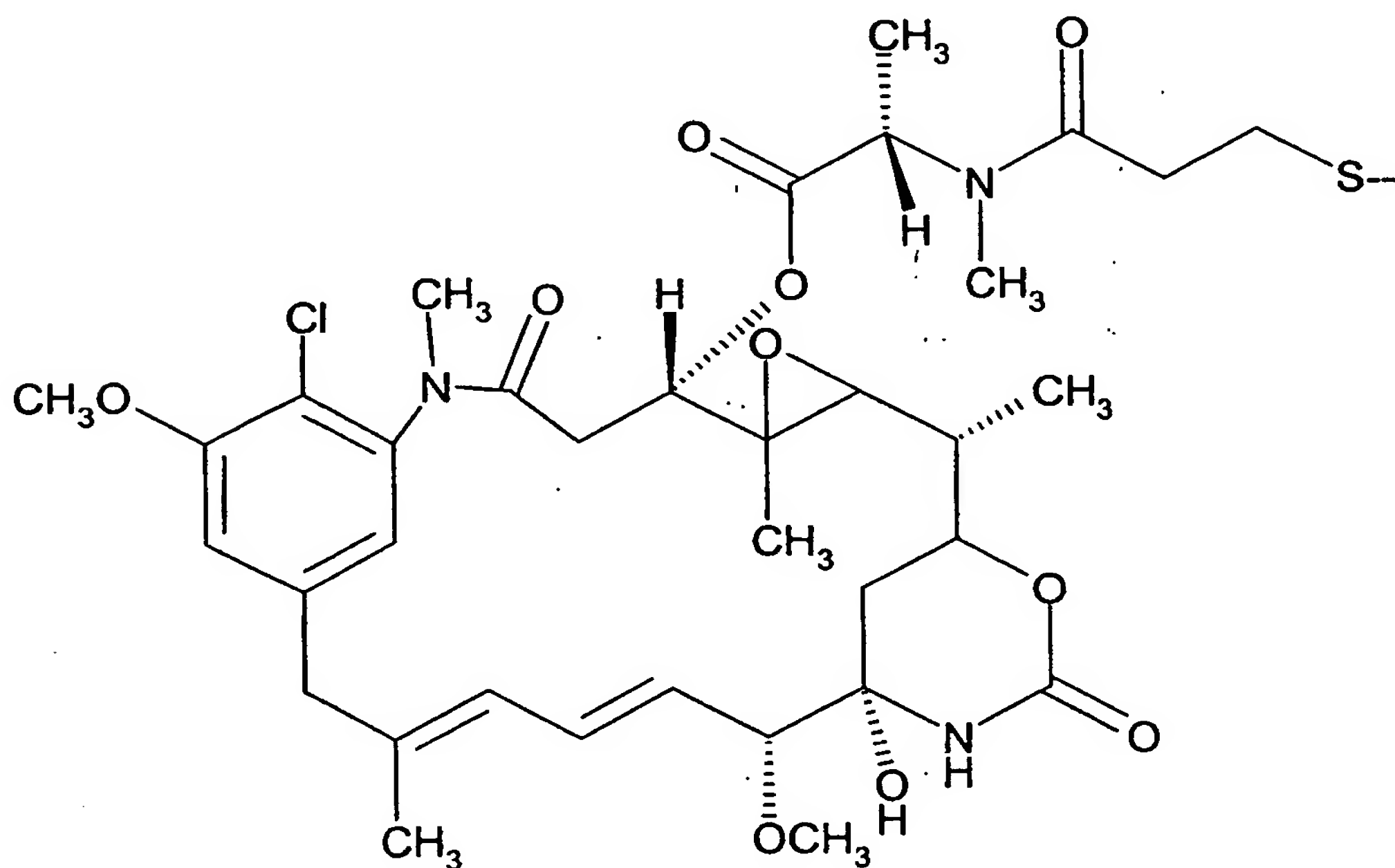
has binding specificity for an epitope within said sequence. More preferably, the antibody molecule specifically binds to a peptide having the amino acid sequence SEQ ID NO: 2, even more preferably having the amino acid sequence SEQ ID NO: 3.

- 5 Preferably, the antibody molecule in said conjugate is the monoclonal antibody VFF-18 (DSM ACC2174) or a recombinant antibody having the complementary determining regions (CDRs) of VFF-18. More preferably, the said antibody comprises light chains having the amino acid sequence SEQ ID NO: 4, or, alternatively, SEQ ID NO: 8, and heavy chains having the amino acid sequence SEQ ID NO: 6.

10

The maytansinoid is preferably linked to the antibody by a disulfide moiety and has the formula

Formula (IV)



15

wherein the link to the antibody is through the sulfur atom shown in formula IV to a second sulfur atom present in the antibody molecule. To create such a sulfur atom available for bonding, an antibody molecule may be modified by introduction of a suitable linker as outlined above. Preferably, the maytansinoid is linked to the antibody molecule through a -

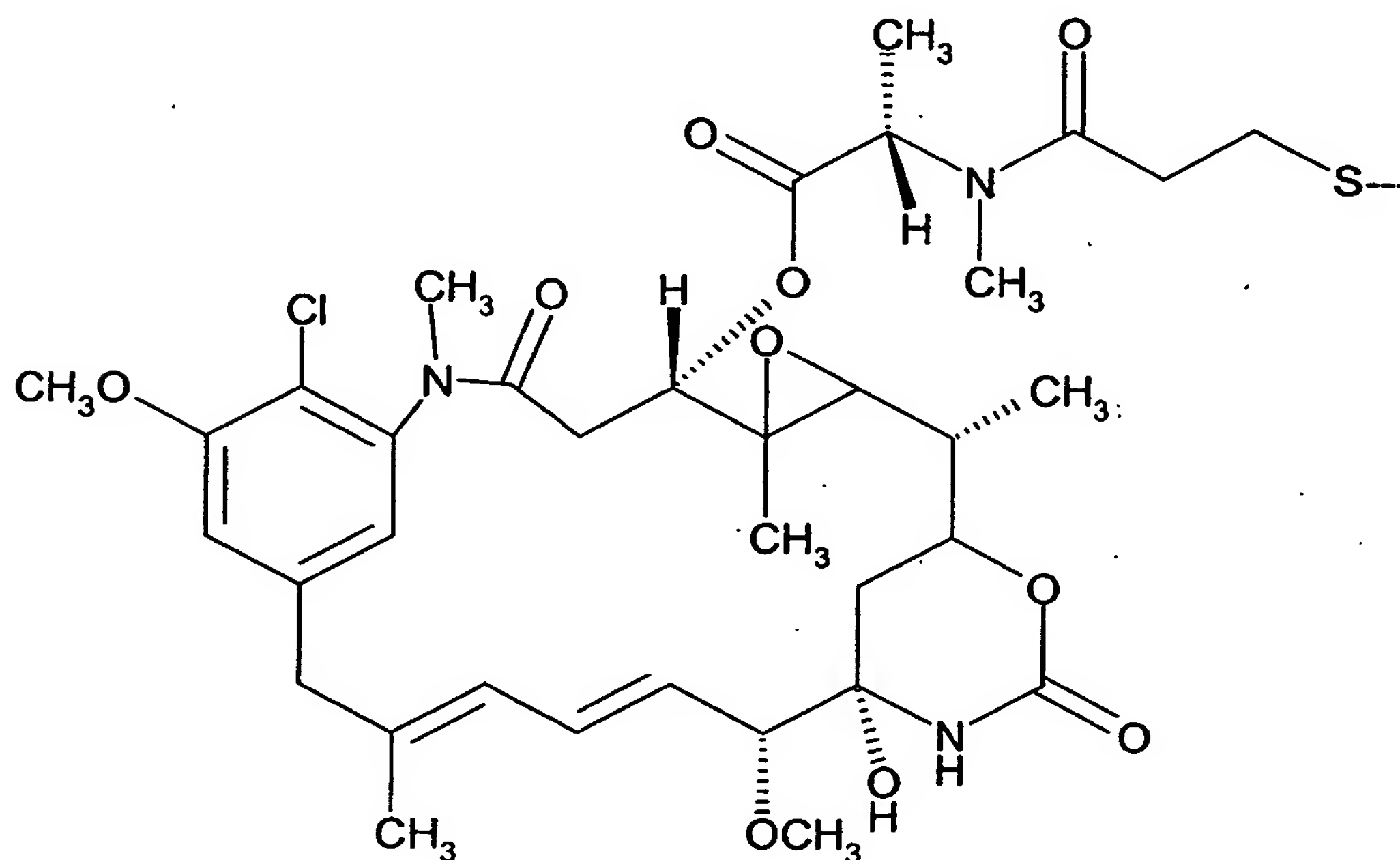
20 S-CH₂CH₂-CO-, a -S-CH₂CH₂CH₂CH₂-CO-, or a -S-CH(CH₃)CH₂CH₂-CO- group. The sulfur atom in such a linker group forms the disulfide bond with the maytansinoid, while

the carbonyl function may be bonded to an amino function present on the side chain of an amino acid residue of the antibody molecule.

That way, one or more maytansinoid residues may be linked to an antibody molecule.

5 Preferably, 3 to 4 maytansinoid residues are linked to an antibody molecule.

Most preferred is a conjugate of a CD44v6 specific antibody molecule and a maytansinoid, wherein the antibody comprises light chains having the amino acid sequence SEQ ID NO: 4, and heavy chains having the amino acid sequence SEQ ID NO: 6, and wherein the
10 maytansinoid has the formula



(Formula IV)

and is linked to the antibody through a disulfide bond. Preferably, the linking group is -S-
15 CH₂CH₂CH₂CH₂-CO- or -S-CH(CH₃)CH₂CH₂-CO-, and the number of maytansinoid residues bound per antibody molecule is 3 to 4.

The conjugate or compound of Formula (I), as defined herein, is preferably formulated into a pharmaceutical composition comprising such conjugate or compound, preferably together
20 with a pharmaceutically acceptable carrier, excipient, or diluent.

Suitable pharmaceutically acceptable carriers, diluents, and excipients are well known and can be determined by those of skill in the art as the clinical situation warrants. In general, the conjugate may be formulated in form of a buffered aqueous solution, using a physiologically acceptable buffer like phosphate buffered saline (PBS; 8 g/l NaCl, 0.2 g/l KCl, 1.44 g/l Na₂HPO₄, 0.24 g/l KH₂PO₄ in distilled water, adjusted to pH 7.4 with aqueous HCl), which may contain additional components for solubilisation, stabilisation, and/or conservation, e.g. serum albumin, ethylenediaminetetraacetate (EDTA), benzyl alcohol, or detergents like polyoxyethylenesorbitan monolaurate (Tween 20™). Examples of suitable carriers, diluents and/or excipients include: (1) Dulbecco's phosphate buffered saline, pH about 7.4, containing about 1 mg/ml to 25 mg/ml human serum albumin, (2) 0.9% saline (0.9% w/v NaCl), and (3) 5% (w/v) dextrose. The formulation may also be in form of a freeze-dried powder which may be reconstituted with water or buffer before administration. Such lyophilisates may contain an bulking agent like, for example, mannitol.

15

For clinical treatment of cancer, the compound of Formula (I) according to the invention, in particular the conjugate of a CD44v6 specific antibody molecule and a maytansinoid, will be supplied as solutions that are tested for sterility and for endotoxin levels. Examples of suitable protocols of conjugate administration are as follows. Conjugates may be given weekly for 1 to 6 weeks either as an i.v. bolus, or as a continuous infusion for 5 days. Bolus doses can be given in 50 to 100 ml of isotonic saline to which 5 to 10 ml of human serum albumin has been added. Continuous infusions can be given in 250 to 500 ml of isotonic saline, to which 25 to 50 ml of human serum albumin has been added, per 24 hour period. Dosages will be 10 mg to 400 mg/m² of body surface area per application. The dose applied to the patient per administration has to be high enough to be effective, but must be below the dose limiting toxicity (DLT). In general, a sufficiently well tolerated dose below DLT will be considered maximum tolerated dose (MTD). The expert knows how to determine the MTD (Lambert et al., 1998). For weekly administrations, the MTD can be expected to be in the range of 50 to 200 mg/m². Alternatively, intervals between applications may be longer, e.g. two to four weeks, preferably three weeks. In this case, the MTD can be expected to be in the range of 100 to 300 mg/m². Alternatively, application may be in 5 daily doses, followed by a break of several weeks after which treatment may be repeated. In this case, the MTD per administration can be expected to be lower than 100

30

mg/m². For example, conjugates can be administered as a single i.v. infusion with a rate of 3 mg/min every 21 days.

„Chemotherapeutic agent“, in the context of this invention, shall mean a chemical
 5 compound which inhibits or kills growing cells and which can be used or is approved for use in the treatment of cancer. As the compound of Formula (I), in particular the conjugate of a CD44v6 specific antibody molecule and a maytansinoid itself is a chemotherapeutic agent in that sense, it is understood that the present invention relates to the combination of such a compound of Formula (I) or conjugate with a second, structurally different
 10 chemotherapeutic agent. Preferably, the chemotherapeutic agent to be combined with a compound of Formula (I), or conjugate as defined above, is not itself an immunoconjugate. Preferred chemotherapeutic agents for such a combination are cytostatic agents which prevent, disturb, disrupt or delay cell division at the level of nuclear division or cell plasma division. Preferably, the chemotherapeutic agent is an anti-mitotic agent, in particular it is a
 15 spindle poison acting by interfering with microtubule function, causing mitotic arrest. Such agents may be classified in two groups, those like taxanes that stabilise microtubule lattices or those, among them the vinca alkaloids, that preferentially form alternate lattice contact and polymers at microtubule ends and thus destabilise microtubules (Future Oncology 6: 1421-1456, 2002; Goodsell, 2000). Both types of spindle poisons are comprised in the
 20 present invention.

Hence, preferred chemotherapeutic agents are those which bind to tubulin. Preferred agents stabilising microtubules are taxanes, in particular docetaxel or paclitaxel, and epothilones, in particular epothilone A, B, C, D, E, and F. Preferred agents which destabilise
 25 microtubules are vinca alkaloids, in particular vinblastine, vincristine, vindesine, vinflunine, and vinorelbine.

Taxanes are anti-mitotic agents isolated from yew trees of the genus *taxus* and their naturally occurring, semi-synthetically, or synthetically obtained derivatives (Future
 30 Oncology 6: 1421-1456, 2002; The Oncologist 5: 345-346, 2000). Paclitaxel (Taxol[®], Suffness [Ed.], 1995; Wani et al., 1971; Holton et al., 1994; Runowicz et al., 1993) is an approved anti-cancer drug formulated as a non-aqueous solution (in polyoxyethylated castor oil/ethanol) intended for dilution with a suitable parenteral fluid for intravenous

infusion at doses of 15-275 mg/m² applied as 1-, 6-, or 24 hour infusions. For combination therapy with another chemotherapeutic drug, an infusion at a dose of 135 or 175 mg/m² over a period of 3 or 24 hours is recommended. Docetaxel (Taxotere[®]; EP 0 253 738; US 4,814,470; Mangatal et al., 1989; Denis et al., 1991; Burris et al., 1993) is also an approved
 5 anti-neoplastic taxane drug. Recommended administration is at a dose of 60 to 100 mg/m² infused intravenously over a period of 1 week. Another taxane useful in the context of the present invention is RPR-116258A (Goetz et al., 2001). Other taxanes known in the art which are useful for cancer treatment may be used for the present invention as well. Such taxanes are disclosed for example in WO 01/70718, WO 01/57028, WO 01/57027, WO
 10 01/56564, WO 01/57030, WO 01/57029, WO 01/57031, WO 01/56565, WO 01/57032, WO 01/27115, WO 01/55126, WO 00/50059, US 6,002,023, WO 99/52887, US 5,998,656, WO 99/32473, US 5,892,063, WO 99/14209, US 5,763,477, WO 98/14187, WO 98/08833, WO 98/02426, US 5,705,508, US 5,703,247, WO 97/43291, WO 97/10234, WO 97/09979, EP 747 372, WO 96/21658, GB 2296239, WO 96/14308, WO 96/03394, EP 693 485, GB
 15 2289277, WO 95/25728, WO 95/24402, WO 95/11020, EP 639 577, WO 94/27984, US 5,367,086, WO 94/20088, WO 95/07900, WO 94/21651, WO 94/21252, WO 94/21251, WO 94/21250, EP 617 034, WO 94/17052, WO 94/17050, WO 94/25449, WO 94/15599, EP 604 910, WO 94/12484, WO 94/10997, WO 94/08984, EP 668 762, WO 94/11362, WO 94/01425, WO 93/23389, EP 534 709, EP 534 708, and EP 534 707.

20

Other compounds that can be used in the invention are those that act through a taxane mechanism. Compounds that act through a taxane mechanism include compounds that have the ability to exert microtubule-stabilizing effects and cytotoxic activity against
 25 proliferating cells, such as tumor cells or other hyperproliferative cellular diseases. Such compounds include, for example, epothilone compounds, such as, for example, epothilone A, B, C, D, E, F, BMS-247550, BMS- 310705, and derivatives thereof. Epothilone compounds are spindle poisons produced by certain myxobacteria and their naturally occurring, semi-synthetically, or synthetically obtained derivatives. Epothilone compounds and derivatives thereof are known in the art and are described, for example, in U. S. Patent
 30 Nos. 6,121,029, 6,117,659, 6,096,757, 6,043,372, 5,969,145, 5,886,026, WO 97/19086, WO 98/08849, WO 98/22461, WO 98/25929, WO 98/38192, WO 99/01124, WO 99/02514, WO 99/03848, WO 99/07692, WO 99/27890, and WO 99/28324. Particularly preferred is epothilone B (CAS No. 152044-54-7; WO 98/25929; White et al., 1999;

Valluri et al., 2001; Muhlradt et al., 1997; Chou et al., 1998; Chen et al., 2000) which may be applied at doses between 0.3 and 3.6 mg/m², more preferably between 0.3 and 2.5 mg/m². Also preferred is the epothilone B analogue BMS-247550 disclosed by Yamaguchi et al., 2002, and Lee et al., 2001. Also preferred is the epothilone A analogue BMS-310705 (Vite et al., 2002; Mekhail et al., 2002). Also preferred is epothilone A, the synthesis of which is described by Zhu et Panek, 2000, and epothilone E, the synthesis of which is described by Nicolaou et al., 1999. Also preferred is epothilone D (US 6,204,388; US 6,303,342; Wang et al., 2001; Chou et al., 1998) which may be applied at a dose of 10-20 mg/m².

10

Vinca alkaloids are spindle poisons produced by plants of the genus *catharanthus* (formerly *vinca* Linn.), in particular *catharanthus roseus*, and their naturally occurring, semi-synthetically, or synthetically obtained derivatives. Vinblastine, e.g. in the form of its sulfate salt, is an approved anti-cancer drug (Gorman et al., 1959; US 3,097,137; US 3,225,030; Sieber et al., 1976; Lu et Meistrich, 1979; Muhtadi et Afifi, 1992). The sulfate salt may be formulated as pure substance which is dissolved in physiological saline before administration and may be applied as a intravenous bolus injection at a dose of 3 to 18.5 mg/m², preferably 5.5 to 7.5 mg/m² once weekly. Vincristine, e.g. in the form of its sulfate, is also an approved anti-cancer drug (Neus et al., 1964; Sieber et al., 1976; Owellen et Donigian, 1972; Burns, 1972). The sulfate may be formulated as a solid preparation containing an equal amount of lactose as an excipient and dissolved in isotonic saline before administration. 1 to 2 mg/m² of the drug may be applied per week as an intravenous bolus application. Vindesine is a synthetic derivative of vinblastine and also an approved chemotherapeutic agent (DE 2415980; Burnett et al., 1978; Owellen et al., 1977; Krivit et al., 1979). Vindesine sulfate may be formulated as a powder formulation with mannitol as an excipient (at a ratio of 1:5) and dissolved in water or isotonic saline before administration. 2-4 mg/m² may be applied as a weekly intravenous bolus injection. Vinorelbine is a semi-synthetic vinca alkaloid also approved for cancer therapy (US 4,307,100; Mangeney et al., 1979; Rahmani et al., 1987; Marty et al., 1989). It may be formulated as a vinorelbine bis[(R,R)-tartrat] in water at a concentration of 10 mg/ml, diluted with isotonic saline or 5% glucose solution before administration, and intravenously infused at a dose of 20 to 30 mg/m² per week. Vinflunine (CAS 162652-95-1; Decosterc et al., 1999) and navelbine (Van-den-Berge et al., 1993) may also be used for

30

the present invention. In the art, further vinca alkaloids are known which may be used in connection with the present invention (WO 99/62912; WO 98/45301; US 5,369,111; US 5,888,537; US 5,891,724; 5,676,978; US 4,096,148).

5 Further microtubule-destabilising agents to be used in the context of the present invention are 5,6-dihydroindolo[2,1-a]isoquinoline derivatives (Goldbrunner et al., 1997). Particularly suitable is combretastatin A4-phosphate (Horsman et al., 1998), and its derivatives like hydroxphenastatin (Pettit et al., 2000), or AVE 8062 (Ohsumi et al., 1998). Spongistatins like spongistatin 1, 2, 3, 4, 5, 6, 7, 8, or 9, may be also used (EP 0 608 111; 10 EP 0 632 042; EP 0 634 414). Another tubulin antagonist for use in the present invention is E-7010 (CAS 141430-65-1; Hoshi et Castaner, 1993). Other tubulin antagonists which may be used in the context of the present invention are dolastatins like cemadotin hydrochloride (Mross et al., 1996). Mivobulin isethionate may also be used (De-Ines et al., 1994).

15 The compound of formula (I), in particular the conjugate of a CD44v6 specific antibody molecule and a maytansinoid, may well combined with other chemotherapeutic agents like cryptophycins, camptothecins (in particular, camptothecin, topotecan, irinotecan, 9-aminocamptothecin), or epipodophyllotoxins (in particular, etoposide, or teniposide). Also to be used in connection with the present invention are anthracyclines like doxorubicin and 20 daunorubicin. Furthermore, antibiotics like dactinomycin, plicamycin, mitomycin, bleomycin, and idarubicin may be used. Alkylating agents like cyclophosphamide, mechlorethamine, melphalan, chlorambucil, procarbazine, dacarbazine, altretamine, platinum compounds (in particular, cisplatin, carboplatin, oxaliplatin, iproplatin, ormaplatin, tetraplatin), or nitrosureas like carmustine, lomustine, or semustine may be 25 used as well. Also comprised are methotrexate, purine antagonists like mercaptopurine, thioguanine, fludarabine phosphate, cladribine, pentostatin, or pyrimidine antagonists like fluorouracil, cytarabine, or azacytidine.

In the context of this invention, „in combination with“ shall mean that the compound of 30 Formula (I), in particular the conjugate of a CD44v6 specific antibody molecule and a maytansinoid, and the chemotherapeutic agent are administered to the patient in a regimen wherein the patient may profit from the beneficial effect of such a combination. In particular, both drugs are applied to the patient in temporal proximity. In a preferred

embodiment, both drugs are applied to the patient within four weeks (28 days). More preferably, both drugs are administered within two weeks (14 days), more preferred within one week (7 days). In a preferred embodiment, the two drugs are administered within two or three days. In another preferred embodiment, the two drugs are administered at the same day, i.e. within 24 hours. In another embodiment, the two drugs are applied within four hours, or two hours, or within one hour. In another embodiment, the two drugs are administered in parallel, i.e. at the same time, or the two administrations are overlapping in time. For example, they may be infused at the same time, or the infusions may be overlapping in time. If the two drugs are administered at the same time, they may be formulated together in one single pharmaceutical preparation, or they may be mixed together immediately before administration from two different pharmaceutical preparations, for example by dissolving or diluting into one single infusion solution. In another embodiment, the two drugs are administered separately, i.e. as two independent pharmaceutical compositions. In one preferred embodiment, administration of the two drugs is in a way that tumour cells within the body of the patient are exposed to effective amounts of both drugs at the same time. In another preferred embodiment, effective amounts of both drugs are present at the site of the tumour at the same time. In another preferred embodiment, effective amounts of both drugs are present in the body of the patient at the same time. The present invention also embraces the use of further agents, which are administered in addition to the combination as defined. This could be, for example, one or more further chemotherapeutic agent(s). It could also be one or more agent(s) applied to prevent, suppress, or ameliorate unwanted side effects of any of the other drugs given. For example, a cytokine stimulating proliferation of leukocytes may be applied to ameliorate the effects of leukopenia or neutropenia.

25

Dose, route of administration, application scheme, repetition and duration of treatment will in general depend on the nature of the disease (type, grade, and stage of the tumor etc.) and the patient (constitution, age, gender etc.), and will be determined by the medical expert responsible for the treatment. With respect to the possible doses for the components of the disclosed combination which are described above, it is clear that the medical expert responsible for the treatment will carefully monitor whether any dose-limiting toxicity or other severe side effects occur and undertake the necessary steps to manage those.

30

The present invention is of particular advantage for the treatment of squameous cell carcinomas expressing CD44 antigen, preferably CD44v6. It is in particular suitable for head and neck squameous cell carcinoma, esophagus squameous cell carcinoma, lung squameous cell carcinoma, skin squameous cell carcinoma, or cervix squameous cell carcinoma. Furthermore, it is of particular advantage for the treatment of adenocarcinomas expressing CD44 antigen, preferably CD44v6. It is in particular suitable for breast adenocarcinoma, lung adenocarcinoma, pancreas adenocarcinoma, colon adenocarcinoma, or stomach adenocarcinoma. Besides treatment of clinically apparent malignant disease, therapeutic application according to the invention may be particularly advantageous as an adjuvant to surgical intervention, to treat minimal residual disease.

In a further aspect, the present invention relates to a pharmaceutical composition comprising a compound $A(LB)_n$ or conjugate as defined herein, together with a chemotherapeutic agent as defined herein, and optionally further comprising one or more pharmaceutically acceptable carrier(s), diluent(s), or excipient(s).

In a further embodiment, the present invention relates to a kit comprising, in separate pharmaceutical compositions, a compound $A(LB)_n$ as defined before, in particular a conjugate of a CD44v6 specific antibody molecule and a maytansinoid, and a chemotherapeutic agent as herein defined.

In a further embodiment, the present invention relates to the use of a chemotherapeutic agent for the preparation of a pharmaceutical composition for the treatment of cancer, wherein said chemotherapeutic agent is used or is for use in combination with a compound of Formula $A(LB)_n$ as herein defined, in particular a conjugate of a CD44v6 specific antibody molecule and a maytansinoid. Preferably, the chemotherapeutic agent is a taxane, an epothilone, a vinca alkaloid, or another tubulin antagonist, a platinum compound, a camptothecin, a cryptophycin, a dolastatin, an epipodophyllotoxin, an alkylating agent, a purine antagonist, a pyrimidine antagonist, or a DNA intercalator. In preferred embodiments, the chemotherapeutic agent is docetaxel, paclitaxel, RPR-116258A, epothilone A, B, C, D, E, or F, BMS-247550, BMS-310705, vinblastine, vindesine, vincristine, vinorelbine, combretastatin A4-phosphate, hydroxphenastatin, AVE 8062, spongistatin 1, 2, 3, 4, 5, 6, 7, 8, or 9, E-7010, dolastatin, cemadotin hydrochloride,

mivobulin isethionate, cryptophycin, camptothecin, topotecan, irinotecan, 9-aminocamptothecin, cisplatin, carboplatin, oxaliplatin, iproplatin, ormaplatin, tetraplatin, etoposide, teniposide, doxorubicin, daunorubicin, dactinomycin, plicamycin, mitomycin, bleomycin, idarubicin, cyclophosphamide, mechlorethamine, melphalan, chlorambucil, procarbazine, dacarbazine, altretamine, carmustine, lomustine, semustine, methotrexate, mercaptopurine, thioguanine, fludarabine phosphate, cladribine, pentostatin, fluorouracil, cytarabine, or azacytidine.

In a further aspect, the present invention relates to a method of treatment of cancer, wherein an effective amount of a chemotherapeutic agent, as defined herein, is administered to a patient in need thereof in combination with a compound of Formula A(LB)_n, as herein defined, in particular a conjugate of a CD44v6 specific antibody molecule and a maytansinoid.

References

- Aguiar DJ, Knudson W, and Knudson CB. Internalization of the hyaluronan receptor CD44 by chondrocytes. *Exp.Cell.Res.* 252: 292-302, 1999.
- Aujame L, Geoffroy F, Sodoyer R. High affinity human antibodies by phage display. *Hum. Antibodies* 1997;8(4):155-68, 1997.
- Bazil V and Horejsi V. Shedding of the CD44 adhesion molecule from leukocytes induced by anti-CD44 monoclonal antibody simulating the effect of a natural receptor ligand. *J. Immunol.* 149 (3):747-753, 1992.
- Blakey and Thorpe, *Antibody, Immunoconjugates and Radiopharmaceuticals*, 1: 1-16, 1988.

Blattler et al, Biochem. 24: 1517-1524, 1985.

Breitling F, Duebel S: Recombinant Antibodies. John Wiley, New York, 1999.

5 Burnett et al., J. Med. Chem. 21: 88, 1978.

Burns, Anal. Prof. Drug Subs. 1: 463-480, 1972.

Burris et al. J. Clin. Oncol. 11: 950, 1993.

10

Carlsson et al, Biochem. J. 173: 723-737, 1978.

Catty D. Antibodies. Oxford IR Press, 1988.

15 Chari RVJ, Martell BA, Gross JL, Cook SB, Shah SA, Blättler WA, McKenzie SJ, Goldmacher VS. Immunoconjugates containing novel maytansinoids: promising anticancer drugs. Cancer Research 52: 127-31, 1992.

20 Chari RVJ, Derr SM, Steeves RM, Widdison WC: Dose-response of the anti-tumor effect of HUN901-DM1 against human small cell lung cancer xenografts. Proceedings of the American Association of Cancer Research (April 1-5, 2000) 41:(April 1-5) Abs 4405, 2000.

Chen et al., Proc. Annu. Meet. Am. Assoc. Cancer Res. 41: Abs 4578, 2000.

25

Chothia and Lesk. J. Mol. Biol. 196: 901-917, 1987.

Chou et al., Proc. Natl. Acad. Sci. USA 95(16): 9642-9647, 1998.

30 Decosterc et al., Anti-Cancer Drugs 10(6): 537-543, 1999.

De-Ines et al., Cancer Res. 54(1): 75-84, 1994.

Denis et al. J. Org. Chem. 56: 6939, 1991.

Frank et al. Methods Enzymol. 154: 221-249, 1984.

5 Gait, M.J., Oligonucleotide Synthesis. A Practical Approach. IRL Press, Oxford, UK, 1984.

Goetz A D et al., Proc. Am. Soc. Clin. Oncol., 20:Pt 1 (Abs 419), 2001.

Goldbrunner et al., J. Med. Chem. 40(22): 3524-3533, 1997.

10

Goldmacher et al., J. Immunol. 135: 3648-3651, 1985.

Goldmacher et al., J. Cell Biol. 102: 1312-1319, 1986.

15 Goodsell, The Oncologist 5: 345-346, 2000.

Gorman et al., J. Am. Chem. Soc. 81: 4745-4754, 1959.

Güssow D, Seemann G. Humanization of monoclonal antibodies. Methods Enzymol. 203:
20 99-121, (1991).

Guo YJ, Liu G, Wang X, Jin D, Wu M, Ma J, and Sy MS. Potential use of soluble CD44
in serum as indicator of tumor burden and metastasis in patients with gastric or colon
cancer. Cancer Res 54 (2): 422-426, 1994.

25

Harlow L D. Antibodies. Cold Spring Harbor Lab., 1988.

Hayden et Mandecki. Gene synthesis by serial cloning of oligonucleotides. DNA 7(8): 571-
7, 1988.

30

Heider, K.-H., Hofmann, M., Horst, E., van den Berg, F., Ponta, H., Herrlich, P., and Pals,
S.T. A human homologue of the rat metastasis-associated variant of CD44 is expressed in
colorectal carcinomas and adenomatous polyps. J. Cell Biol. 120: 227-233, 1993.

- Heider KH, Mulder JWR, Ostermann E, Susani S, Patzelt E, Pals ST, Adolf GRA. Splice variants of the cell surface glycoprotein CD44 associated with metastatic tumor cells are expressed in normal tissues of humans and cynomolgus monkeys. Eur. J. Cancer 31A: 2385-2391, 1995.
- Heider KH, Sproll M, Susani S, Patzelt E, Beaumier P, Ostermann O, Ahorn H, Adolf GRA. Characterization of a high affinity monoclonal antibody specific for CD44v6 as candidate for immunotherapy of squamous cell carcinomas. Cancer Immunology Immunotherapy 43: 245-253, 1996.
- Hofmann, M., Rudy, W., Zöller, M., Tölg, C., Ponta, H., Herrlich P., and Günthert, U. CD44 splice variants confer metastatic behavior in rats: homologous sequences are expressed in human tumor cell lines. Cancer Res. 51: 5292-5297, 1991.
- Holton et al. J. Am. Chem. Soc. 116: 1597, 1599, 1994.
- Horsman et al., Proc. Annu. Meet. Am. Assoc. Cancer Res. 39: Abs 1142, 1998.
- Hoshi et Castaner, Drugs Future 18(11): 995-996, 1993.
- Johnson S, Bird R E. Construction of single-chain derivatives of monoclonal antibodies and their production in *Escherichia coli*. Methods Enzymol. 203: 88-98, 1991.
- Kabat E. A., Wu T. T., Perry H. M., Gottesman K. S. and Foeller C. Sequences of Proteins of Immunological Interest (5th Ed.). NIH Publication No. 91-3242. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Bethesda, MD, 1991.
- Klotz et al, Arch. Biochem. Biophys. 96: 605, 1962.
- Koopman, G., Heider, K.-H., Horts, E., Adolf, G. R., van den Berg, F., Ponta, H., Herrlich, P., Pals, S. T. Activated human lymphocytes and aggressive Non-Hodgkin's lymphomas

express a homologue of the rat metastasis-associated variant of CD44. *J. Exp. Med.* 177: 897-904, 1993.

5 Kortt AA, Dolezal O, Power BE, Hudson PJ. Dimeric and trimeric antibodies: high avidity scFvs for cancer targeting. *Biomol. Eng.* 18(3), 95-108, 2001.

Kreitman R J Hansen H J, Jones A L, FitzGerald D J P, Goldenberg D M, Pastan I. *Pseudomonas* exotoxin-based immunotoxins containing the antibody LL2 or LL2-Fab' induce regression of subcutaneous human B-cell lymphoma in mice. *Cancer Res.* 53: 819-825,
10 1993.

Krivit et al., *Cancer Chemother. Pharmacol.* 2: 267, 1979.

15 Lambert et al, *Biochem.* 22: 3913-3920, 1983.

Lambert JM, Derr SM, Cook S, Braman G, Widdison W, Chari RVJ. Pharmacokinetics, in vivo stability, and toxicity of the Tumor-activated prodrug, C242-DM1, a novel colorectal cancer agent. *Proceedings of the American Association of Cancer Research* 39: Abs 3550, 1998.

20 Lee et al., *Clin Cancer Res* 7(5): 1429-1437, 2001.

Liu et al., *Biochem.* 18: 690, 1979.

25 Liu C, Tadayoni BM, Bourret LA, Mattocks KM, Derr SM, Widdison WC, Kedersha NL, Ariniello PD, Goldmacher VS, Lambert JM, Blättler WA, Chari RVJ. Eradication of large colon tumor xenografts by targeted delivery of maytansinoids. *Proc. Natl. Acad. Sci. USA* 93: 8618-23, 1996.

30 Lu et Meistrich, *Cancer Res.* 39: 3575, 1979.

Mangeney et al., *Tetrahedron* 35: 2175, 1979.

Mangatal et al. Tetrahedron 45: 4177, 1989.

Martin S, Jansen F, Bokelmann J, and Kolb H. Soluble CD44 splice variants in metastasizing human breast cancer. Int. J. Cancer 74 (4): 443-445, 1997.

5

Marty et al., Nouv. Rev. Fr. Hematol. 31: 77-84, 1989.

Mekhail et al., Proceedings of the American Society for Clinical Oncology, 21:1(Abs 408), 2002.

10

Mross et al., Onkologie 19(6): 490-495, 1996.

Muhlradt et al., Cancer Res 57(16): 3344-3346, 1997.

15 Muhtadi et Afifi, Analytical Profiles of Drug Substances and Excipients 21: 611-658, Brittain (Ed.), Academic Press, San Diego, 1992.

Neus et al., J. Am. Chem. Soc. 86: 1440, 1964.

20 Nicolaou et al., Bioorg. Med. Chem. 7(5): 665-697, 1999.

Ohsumi et al., J. Med. Chem. 41(16): 3022, 1998.

Owellen et Donigian, J. Med. Chem. 15: 894, 1972.

25

Owellen et al., Cancer Res. 37: 2603, 1977.

Pettit et al., J. Med. Chem. 43(14): 2731-2737, 2000.

30 Rahmani et al., Cancer Res. 47: 5796-5799, 1987.

Runowicz et al. Cancer 71: 1591-1596, 1993.

- Screaton, G.R., Bell, M.V., Jackson, D.G., Cornelis, F.B., Gerth, U., and Bell, J. I. Genomic structure of DNA encoding the lymphocyte homing receptor CD44 reveals at least 12 alternatively spliced exons. *Proc. Natl. Acad. Sci. U.S.A.* 89: 12160-12164, 1992.
- 5 Shin S-U, Morrison S L. Production and properties of chimeric antibody molecules. *Methods Enzymol.* 178: 459-476, 1989.
- Sieber et al., *Cancer Treat. Rep.* 60: 127, 1976.
- 10 Sliutz G, Tempfer C, Winkler S, Kohlberger P, Reinhaller A, and Kainz C. Immunohistochemical and serological evaluation of CD44 splice variants in human ovarian cancer. *Br.J.Cancer* 72: 1494-1497,, 1995.
- Stemmer et al. Single-step assembly of a gene and entire plasmid from large numbers of
 15 oligodeoxyribonucleotides. *Gene* 164(1): 49-53, 1995.
- Stroomer JW, Roos JC, Sproll M, Quak JJ, Heider KH, Wilhelm BJ, Castelijns JA, Meyer R, Kwakkelstein MO, Snow GB, Adolf GR, van Dongen GA. Safety and biodistribution of 99mTechnetium-labeled anti-CD44v6 monoclonal antibody BIWA 1 in head and neck
 20 cancer patients. *Clin. Cancer Res.* 6 (8): 3046-55, 2000.
- Suffness [Ed.]. *Taxol®. Science and Applications.* CRC Press, Boca Raton, 1995.
- Tölg, C., Hofmann, M., Herrlich, P., and Ponta, H. Splicing choice from ten variant exons
 25 establishes CD44 variability. *Nucleic Acids. Res.* 21: 1225-1229, 1993.
- Tolcher AW et al. SB-408075, a maytansinoid immunoconjugate directed to the C242 antigen: a phase I pharmacokinetic and biologic correlative study. Poster presented at 11th Symp. on new drugs in cancer therapy (Nov 7-10, 2000 in Amsterdam), 2000.
- 30 Urlaub and Chasin. *Proc. Natl. Acad. Sci. U.S.A.* 77(7): 4216-20, 1980.
- Urlaub et al., *Cell* 33: 405-412, 1983.

Valluri et al., Org Lett 3 (23): 3607-3609, 2001.

Van-den-Berge et al., Anticancer Res. 13(1): 273-277, 1993.

5

Verel et al., Int. J. Cancer 99: 396-402, 2002.

Vite et al., ACS Meeting 2002, 223rd:Orlando(MEDI 18), 2002.

- 10 Wang et al., AACR NCI EORTC Molecular Targets and Cancer Therapeutics 2001, October 29-November 2(Abs #781), 2001.

Wani et al., J. Am. Chem. Soc. 93: 2325, 1971.

- 15 White et al., Org. Lett. 1(9): 1431-1434, 1999.

Winter, G., Griffith, A. D., Hawkins, R. E., Hoogenboom, H. R. Making antibodies by phage display technology. Ann. Rev. Immunol. 12: 433-455, 1994.

- 20 Worrell et al, Anti-Cancer Drug Design 1: 179-184, 1986.

Yamaguchi et al., Cancer Res. 62 (2): 466-471, 2002.

- 25 Ye et al., Gene synthesis and expression in E. coli for pump, a human matrix metalloproteinase. Biochem. Biophys. Res. Commun. 186(1):143-9, 1992.

Zhu et Panek, Org. Lett. 2 (17): 2575-2578, 2000.

Examples

1. Material and Methods

5 1.1. In vitro cell proliferation assay

For determination of viable cells the Cell Titer 96[®] AQueous non-radioactive cell proliferation assay (Promega) was used. 5000 cells per well were seeded into 96-well plates in 90 µl medium without phenole red. Cells were allowed to settle down for 1 to 3 h and then serial dilutions of the immunoconjugate in 10 µl PBS were added. Cells without
10 immunoconjugate served as negative control. Cells were incubated for 4 days at 37°C in a humified 5% CO₂ atmosphere and then 20 µl MTS/ PMS were added according to the manufacturer's recommendation. After additional 1 to 4 h incubation at 37°C the absorbance at 490 nm was recorded using an ELISA plate reader. For each cell line triplicates were analyzed. The percentage of the surviving cell fraction and the IC50 value
15 were calculated using the GraphPad Prism[®] (Version 3.0) software.

1.2. Manufacturing of BIWI 1

Humanised recombinant antibodies BIWA 4 and BIWA 8 which have binding specificity
20 for an epitope within SEQ ID NO: 1 were linked to the maytansinoid DM1 as described below. The conjugate of BIWA 4 with DM1 was designated BIWI 1.

Generation of stably transfected cell lines. The genes coding for the light and heavy chains of BIWA 4, SEQ ID NO: 5 and SEQ ID NO: 7, were ligated into expression vector
25 pAD-CMV1 (WO92/01055; NCBI GenBank Accession No. A32111) or pAD-CMV19 (NCBI GenBank Accession No. A32110). In the second antibody BIWA 8, the light chain was coded by a gene having SEQ ID NO: 9, while the heavy chain was the same as in BIWA 4. Stably transfected cell lines were generated by electroporation as follows. CHO DUX/57ss (dhfr negative mutant of Chinese Hamster Ovary cells, adapted for serum free
30 suspension culture) were used. After trypsinisation and inactivation of trypsin with RPMI-10 (90% RPMI 1640, 10% heat inactivated fetal calf serum), cells were washed once with RPMI-0 (RPMI 1640 without serum), and 1×10^7 cells were resuspended in 0.8 ml RPMI-0. After addition of the linearised DNA (20 µg per plasmid; cotransfection of vectors coding

for light and heavy chain) the cells were electroporated using a Hoefer Electroporator under the following conditions: 1080 μ F, 320 V, 1000 msec, 1 pulse. Cells were allowed to stand for 5 min, and were then diluted to 12500 cells/ml and 2500 cells/ml in alfa-MEM 10d (90% MEM alfa without ribonucleosides and without desoxyribonucleosides (GIBCO BRL), 10% heat inactivated dialysed fetal calf serum). The cells were seeded into 96 well microtiter plates (200 μ l/well, corresponding to 2500 and 500 cells/well respectively). Clones appeared after 10 days. Only the plates with 500 cells/well were followed up (3-6 clones/well). After 14-15 days, supernatants from each well were tested in a κ/γ ELISA. 53 clones were seeded in 12 well plates in alfa-MEM 10d. After 3-6 days (depending on the confluency of the cells) supernatants were tested again in the κ/γ ELISA (serial dilutions) and quantitated using a human IgG1 standard. Cells were frozen and stored in liquid nitrogen. IgG contents of the 53 clones ranged from 12 - 417 ng/ml. 10 clones with the highest expression level were selected and subcloned as follows: Cells of each clone were seeded into 96 well microtiter plates with densities of 1 and 5 cells/well in 100 μ l/well alfa-MEM 10d (1 plate for each clone and each density). 8 days later supernatants were diluted 1:2 and 100 μ l of this dilution tested in the κ/γ ELISA and quantitated using a BIWA 4 preparation as standard. 5 subclones of each clone were transferred to 12 well plates. The IgG content ranged from 1.3 - 908 ng/ml. 14 clones with the highest expression level (384 - 908 ng/ml) were used for amplification with methotrexate as follows: Clones were initially cultured in 25 cm² flasks containing alfa-MEM 10d with 20, 50 and 100 nM methotrexate. After the outgrowth of clones the supernatants were tested in the κ/γ ELISA. In subsequent rounds of amplification the methotrexate concentration was raised up to 2000 nM. Initially the highest expression level ranged from 10.5-14.8 μ g/ml (clone A31/100, 100 nM methotrexate). Further amplification with a methotrexate concentration of 500 nM gave an expression of 19-20 μ g/ml (A31/500).

Purification of antibody. Antibody was purified from cell culture supernatant as follows. Antibody containing tissue culture supernatant was applied onto a 5 ml protein A sepharose column with a flow rate of 80-90 ml/h at 4 °C. After washing with 50 ml binding buffer (0.1 M sodium phosphate pH 7.5), the Ig fraction was eluted with elution buffer (0.1 M glycine-HCl pH 2.7). Absorption at 280 nm was monitored.

Modification of BIWA 4 with SPP to form BIWA 4-SS-Py. BIWA 4 was supplied in liquid form at a concentration of 5 mg/mL in a PBS formulation containing Tween 20. Prior to coupling of DM1 to the MAb the Tween 20 was removed. The MAb solution (40 mL) was diluted 15-fold with 25 mM MES buffer, pH 5.6, containing 50 mM NaCl (MES buffer) and then loaded onto a column (12.5 mL) of Sepharose S equilibrated in MES buffer (flow rate: 100 cm/hr). The column was washed with 10 column volumes of MES buffer. The antibody was eluted with MES buffer containing 400 mM NaCl. The antibody solution was dialysed against 50 mM potassium phosphate buffer, pH 6.5 containing 50 mM NaCl and 2 mM EDTA (Buffer A). The BIWA 4 antibody was modified using SPP ((2-Pyridyl)-5-dithiopentanoic acid N-hydroxy succinimid ester) to introduce dithiopyridyl groups. The MAb in Buffer A (185 mg, 8 mg/mL) was modified with a 7-fold molar excess of SPP in EtOH (5% v/v of MAb solution). The reaction proceeded for 90 minutes at ambient temperature. The reaction mixture was then subjected to gel filtration chromatography through Sephadex G25F (2.6 x 31.5 cm column, 167 mL) equilibrated in Buffer A. MAb-containing fractions were pooled and the degree of modification was determined by measuring the absorbance at 280 nm and the change in absorbance at 343 nm caused by the release of 2-mercaptopyridine by the addition of DTT. The concentration of released 2-mercaptopyridine was calculated using an $\epsilon_{343 \text{ nm}}$ of $8080 \text{ M}^{-1}\text{cm}^{-1}$, and the concentration of MAb was calculated using an $\epsilon_{280 \text{ nm}}$ of $224,000 \text{ M}^{-1}\text{cm}^{-1}$ after the absorbance at 280 nm has been corrected for the contribution from 2-mercaptopyridine. (2-mercaptopyridine $A_{280 \text{ nm}} = A_{343 \text{ nm}} \times 5100/8080$). Recovery of the MAb was 99.6% with 5.5 releasable 2-mercaptopyridine groups linked per MAb molecule.

Conjugation of BIWA 4-SS-Py with DM1. The above modified MAb (184 mg) in Buffer A was conjugated at 2.5 mg MAb/mL using a 1.7-fold molar excess of DM1 over releasable 2-mercaptopyridine groups. DM1 was added in DMA (3% v/v of MAb solution) and the reaction mixture was incubated at ambient temperature for 29 hours. The conjugate was then isolated by gel filtration chromatography on a column of Sephacryl S300 HR equilibrated in PBS (5 x 50 cm column, 980 mL, flow rate of 10 cm/hr). The conjugate eluted as a single peak at the position of monomeric MAb with a small amount of protein eluting earlier. Fractions were assayed for the number of DM1 molecules linked per MAb molecule. (Linked DM1 molecules were determined by measuring the absorbance at both 252 nm and 280 nm). Based on the results, fractions representing 63-77% of the

column volume were pooled. The DM1/MAb ratio in the pooled solution was found to be 3.1 and the yield of conjugated BIWA 4 was 75% based on starting MAb. The conjugate, BIWI 1, was evaluated by SDS-PAGE performed under non-reducing conditions and found to be composed primarily of a monomer species (>95%) with a minor amount (<5%) of dimeric conjugate.

Analysis of *in vitro* binding of BIWI 1. The binding of BIWA 4 antibody and BIWI 1 conjugate to antigen-positive FaDu cells was determined. Cells ($1-2 \times 10^5$) were incubated in 96-well plates with varying concentrations of antibody or conjugate on ice for 1 hour. The test article was washed from the plate and FITC-labeled anti-human IgG was added and the incubation on ice was continued in the dark for 1 hour. After washing, the cells were fixed with 1% paraformaldehyde and analyzed on a fluorescence activated cell sorter (FACS). BIWA 4 antibody binds with an apparent K_D of 1×10^{-9} M and BIWI 1 binds with an apparent K_D of 1.8×10^{-9} M. Thus, conjugation with DM1 alters the binding affinity of the antibody only slightly if at all.

1.3. Efficacy studies in nude mice

In vivo anti-tumor efficacy of BIWI 1 was tested in three nude mouse xenograft models applying antigen-positive human tumors: A431 (ATCC # CRL 1555; epidermoid carcinoma of the vulva), FaDu (ATCC # HTB 43; squamous cell carcinoma of the pharynx), and MDA-MB 453 (ATCC # HTB-131; breast carcinoma). The cells were received from ATCC and cultured in RPMI1640 medium containing 10% fetal calf serum and supplements. 1×10^6 tumors cells were transplanted subcutaneously into the right flank of 6 week old female NMRI-nu/nu mice. Tumor growth was monitored by measuring tumor size. A tumor response was rated as complete response when the tumor completely disappeared at any time after start of treatment. The response was rated as partial response when the tumor volume decreased after treatment but thereafter started regrowing. The tolerability of the treatment was monitored by measuring mouse weight during the observation period.

2. Results and Discussion

2.1. *In vitro* cytotoxicity of BIWI 1

The *in vitro* cytotoxicity of BIWI 1 was evaluated using the antigen-positive cell lines A431 and FaDu, and the antigen-negative cell line A459. Cells were exposed to different concentrations of BIWI 1 for 4 days, then stained with MTS/ PMS and assayed on an ELISA plate reader. The surviving fractions of cells were then calculated using the GraphPad Prism[®] software package. The results are shown in Figure 1. BIWI 1 was effective in killing the antigen-positive A431 cells with an IC_{50} of about 7.6×10^{-8} M and the second antigen-positive cell line, FaDu, with an IC_{50} of about 2.4×10^{-8} M. The antigen-negative cell line, A549, was effected by the conjugate with a surviving fraction of 50% at the highest concentration of BIWI 1 tested (5×10^{-7} M). These results show that BIWI 1 is only slightly more cytotoxic against antigen-positive cells than antigen-negative cells *in vitro*. For comparison, another DM1-antibody conjugate has been shown to be at least 1000fold more cytotoxic against antigen-positive cell as compared to antigen-negative cells (Chari *et al.*, 1992).

2.2. BIWI 1 monotherapy in A431 xenografted nude mice

Mice were randomised into the following treatment groups (treatment/initial mean tumour volume/tumour volume range/number of mice):

- Group 1: Control (PBS) / $185 \pm 217 \text{ mm}^3$ / $19 - 424 \text{ mm}^3$ / 5 mice.
- Group 2: BIWA 4 (21 mg/kg/d) / $133 \pm 115 \text{ mm}^3$ / $42 - 302 \text{ mm}^3$ / 5 mice.
- Group 3: BIWI 1 (2.1 mg/kg/d) / $107 \pm 63 \text{ mm}^3$ / $42 - 205 \text{ mm}^3$ / 5 mice.
- Group 4: BIWI 1 (7 mg/kg/d) / $132 \pm 73 \text{ mm}^3$ / $42 - 205 \text{ mm}^3$ / 5 mice.
- Group 5: BIWI 1 (21 mg/kg/d) / $107 \pm 63 \text{ mm}^3$ / $42 - 205 \text{ mm}^3$ / 5 mice.

Groups of 5 mice were treated with 2.1 mg/kg/d BIWI 1, 7 mg/kg/d BIWI 1, 21 mg/kg/d BIWI 1, and 21 mg/kg/d control antibody, respectively. Treatment consisted of i.v. injections of BIWI 1 given on five consecutive days, starting at day 1. The average tumor

volume of each group during the observation period is shown in Figure 2. Tumors treated with control antibody showed similar growth as untreated tumors, the tumor volume doubling time was approximately 5 days. In animals treated either with 7 mg/kg/d BIWI 1 or 21 mg/kg/d BIWI 1 all tumors responded completely and disappeared around day 17. No tumor regrowth was observed until the end of the observation period (day 134). Tumors treated with 2.1 mg/kg/d responded completely in 3/5 cases with no tumor regrowth until day 134. The remaining 2 tumors showed a partial response but ultimately regrew. These results show that BIWI 1 induces a dose-dependent anti-tumor response in A431 xenografted nude mice, with complete and long-lasting responses from 2.1 mg/kg/d BIWI 1 to 21 mg/kg/d BIWI 1. Unconjugated control antibody shows no anti-tumor effect.

See Figure 2.

2.3. BIWI 1 monotherapy in FaDu xenografted nude mice

Mice were randomised into the following treatment groups (treatment/initial mean tumour volume/tumour volume range/number of mice):

- Group 1: Control (PBS) / $142 \pm 82 \text{ mm}^3$ / 34 – 268 mm^3 / 8 mice.
- Group 2: BIWA 4 (21 mg/kg/d) / $134 \pm 86 \text{ mm}^3$ / 42 – 268 mm^3 / 6 mice.
- Group 3: BIWI 1 (2.1 mg/kg/d) / $149 \pm 96 \text{ mm}^3$ / 50 – 268 mm^3 / 6 mice.
- Group 4: BIWI 1 (7 mg/kg/d) / $132 \pm 97 \text{ mm}^3$ / 42 – 268 mm^3 / 6 mice.
- Group 5: BIWI 1 (21 mg/kg/d) / $129 \pm 74 \text{ mm}^3$ / 50 – 231 mm^3 / 6 mice.

Groups of 6 mice were treated with 2.1 mg/kg/d BIWI 1, 7 mg/kg/d BIWI 1, 21 mg/kg/d BIWI 1, and 21 mg/kg/d control antibody, respectively. Treatment consisted of i.v. injections of BIWI 1 given on five consecutive days, starting at day 1. The average tumor volume of each group during the observation period is shown in Figure 3. Tumors treated with control antibody and 2.1 mg/kg/d BIWI 1 showed similar growth as untreated tumors, the tumor volume doubling time was approximately 5 days. In animals treated with 21 mg/kg/d BIWI 1 all tumors responded completely and disappeared around day 24. No tumor regrowth was observed until the end of the observation period (day 107). 1/6 tumors treated with 7 mg/kg/d BIWI 1 responded completely, 3/6 tumors showed partial responses.

The remaining 2 tumors grew similar to untreated control tumors. These results show that BIWI 1 induces a dose-dependent anti-tumor response in FaDu xenografted nude mice, with complete and long-lasting responses from 7 mg/kg/d BIWI 1 to 21 mg/kg/d BIWI 1. Unconjugated control antibody shows no anti-tumor effect.

5

See Figure 3.

2.4. BIWI 1 monotherapy in MDA-MB 453 xenografted mice

10

Groups of 6 mice were treated with 6.25 mg/kg BIWI 1, 12.5 mg/kg BIWI 1, and 25 mg/kg BIWI 1, respectively. Treatment consisted of i.v. injections of BIWI 1 given weekly for four weeks. The average tumor size at start of treatment was $246 \pm 79 \text{ mm}^3$ (PBS), $216 \pm 85 \text{ mm}^3$ (6.25 mg/kg BIWI 1), $188 \pm 79 \text{ mm}^3$ (12.5 mg/kg BIWI 1), and $207 \pm 96 \text{ mm}^3$ (25 mg/kg BIWI 1), respectively. The average tumor volume of each group during the observation period is shown in Figure 4. The initial tumor volume doubling time of the control tumors was approximately 5 days. In animals treated with 25 mg/kg BIWI 1 all tumors responded completely and disappeared around day 22 after start of treatment. No tumor regrowth was observed until the end of the observation period (day 64). Tumors treated with 12.5 mg/kg or 6.25 mg/kg responded completely in 5/6 cases in each dose group, and 4 animals of each group stayed tumor free until the end of the experiment. These results show that BIWI 1 induces anti-tumor responses in MDA-MB 453 xenografted nude mice when given once a week over a period of four weeks, with complete and long-lasting responses from 6.25 mg/kg BIWI 1 to 25 mg/kg BIWI 1.

25

See Figure 4.

2.5. Tolerability of BIWI 1 monotherapy in nude mice

30

The tolerability of BIWI 1 monotherapy was determined by monitoring mouse weight during the whole duration of the experiment in the 2 models. The maximum observed average weight loss per group was 6% in FaDu xenografted mice treated with 21 mg/kg/d

BIWI 1 (Figure 5). The weight loss started around day 3 of treatment and lasted until day 10, thereafter animals regained weight and behaved similar as control animals. In all other dose groups weight loss was similar to vehicle control (PBS). An average weight loss of 6% or less in all treatment groups indicates good tolerability of BIWI 1 treatment at the given doses in nude mice. As BIWI 1 does not cross-react with mouse CD44v6, only antigen-independent effects such as toxicity caused by free DM1 can be monitored in this experiment.

See Figure 5.

2.6. BIWI 1 combination therapy with paclitaxel in the FaDu xenograft model

Mice were randomised into the following treatment groups (treatment/initial mean tumor volume/tumor volume range/number of mice):

- Group 1: Control (PBS)/ 197 mm³ / 65 – 402 mm³ / 8 mice
- Group 2: Paclitaxel / 182 mm³ / 65 – 302 mm³ / 8 mice
- Group 3: BIWI 1 / 208 mm³ / 65 – 382 mm³ / 8 mice
- Group 4: BIWI 1 + paclitaxel/ 159 mm³ / 79 – 335 mm³ / 7 mice

Treatment consisted either of five i.v. injections of BIWI 1 (7 mg/kg corresponding to 100 µg/kg DM1) at day 1, 3, 5, 8, 10, or of six i.p. injections of paclitaxel (10 mg/kg) at day 2, 4, 6, 9, 11, 13, respectively, or a combination thereof. Control animals were treated with PBS.

25

The mean relative tumor volumes during treatment are shown in Figure 6, individual tumor volumes are shown in Figure 7. Monotherapy with paclitaxel resulted in a tumor growth delay compared to PBS treated animals. BIWI 1 monotherapy resulted in a partial response in 3/ 8 animals which showed a clear delay in tumor growth compared to PBS-treated animals. In the combination group all tumors responded to treatment and showed clearly delayed tumor growth compared to control tumors. One tumor completely disappeared during treatment and the animal remained tumor free until the end of the observation period. These experiments demonstrate a markedly increased anti-tumor efficacy of a

combination of BIWI 1 with paclitaxel compared to the respective monotherapies, indicating a synergistic effect of the combination therapy.

See Figures 6 and 7.

5

2.7. Tolerability of BIWI 1 combination therapy in nude mice

The tolerability of the treatment was determined by monitoring mouse weight during the whole duration of the experiment. The maximum observed average weight loss per group was less than 5% in the combination group. In all other dose groups basically no weight loss upon treatment was observed. An average weight loss of less than 5% indicates good tolerability of the treatment at the given doses in nude mice.

15

Figures

Figure 1: In vitro cytotoxicity of BIWI 1. The antigen-positive cell lines A431 and FaDu and the antigen-negative cell line A549 were used.

20

Figure 2: Efficacy of BIWI 1 treatment in nude mice xenografted with A431 tumors. The average tumor volumes per group with standard deviations are shown, the treatment groups are indicated. The arrow indicates start of treatment (day 1).

25

Figure 3: Efficacy of BIWI 1 treatment in nude mice xenografted with FaDu tumors. The average tumor volumes per group with standard deviations are shown, the treatment groups are indicated. The arrow indicates start of treatment (day 1).

30

Figure 4: Efficacy of BIWI 1 treatment in nude mice xenografted with MDA-MB 453 tumors. The average tumor volumes per group with standard deviations are shown, the treatment groups are indicated. The arrows indicate the treatment days.

Figure 5: Tolerability of BIWI 1 treatment. The average body weight change of all treatment groups in the 2 investigated models is shown. Day 1: start of treatment.

Figure 6: Efficacy of BIWI 1 combination treatment with paclitaxel in nude mice xenografted with FaDu tumors. The average tumor volume per group with standard deviations is shown.

Figure 7: Efficacy of BIWI 1 combination treatment with paclitaxel in nude mice xenografted with FaDu tumors. The individual tumor volumes per group are shown.

Figure 8: BIWI 1 combination treatment with paclitaxel in nude mice xenografted with FaDu tumors. Tolerability of treatment. The average body weight change of all treatment groups is shown. Day 1: start of treatment.

21. Aug. 2002

SEQUENCE LISTING

<110> Boehringer Ingelheim International GmbH
 5 <120> Cytotoxic CD44 Antibody Immunoconjugates
 <130>
 <140>
 10 <141>
 <160> 7
 <170> PatentIn Ver. 2.1
 15 <210> 1
 <211> 42
 <212> PRT
 <213> Homo sapiens
 20 <223> Description of Artificial Sequence: Human CD44 Exon v6 SEQ ID
 NO: 1
 <400> 1
 25 Gln Ala Thr Pro Ser Ser Thr Thr Glu Glu Thr Ala Thr Gln Lys Glu
 1 5 10 15
 Gln Trp Phe Gly Asn Arg Trp His Glu Gly Tyr Arg Gln Thr Pro Arg
 20 25 30
 30 Glu Asp Ser His Ser Thr Thr Gly Thr Ala
 35 40
 35 <210> 2
 <211> 14
 <212> PRT
 <213> Homo sapiens
 40 <223> Description of Artificial Sequence: SEQ ID NO: 2
 <400> 2
 Gln Trp Phe Gly Asn Arg Trp His Glu Gly Tyr Arg Gln Thr
 1 5 10
 45
 <210> 3
 <211> 11
 <212> PRT
 50 <213> Homo sapiens
 <223> Description of Artificial Sequence: SEQ ID NO: 3
 <400> 3
 55 Trp Phe Gly Asn Arg Trp His Glu Gly Tyr Arg
 1 5 10

<210> 4
 <211> 213
 <212> PRT
 <213> Artificial Sequence

5

<220>
 <223> Description of Artificial Sequence: Humanised
 Murine Antibody BIWA 4 Light Chain SEQ ID NO: 4

10

<400> 4
 Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
 1 5 10 15

15

Glu Arg Ala Thr Leu Ser Cys Ser Ala Ser Ser Ser Ile Asn Tyr Ile
 20 25 30

Tyr Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile Tyr
 35 40 45

20

Leu Thr Ser Asn Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser
 50 55 60

Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro Glu
 65 70 75 80

25

Asp Phe Ala Val Tyr Tyr Cys Leu Gln Trp Ser Ser Asn Pro Leu Thr
 85 90 95

30

Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro
 100 105 110

Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr
 115 120 125

35

Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys
 130 135 140

Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu
 145 150 155 160

40

Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser
 165 170 175

45

Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala
 180 185 190

Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe
 195 200 205

50

Asn Arg Gly Glu Cys
 210

55

<210> 5
 <211> 702
 <212> DNA
 <213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Humanised
Murine Antibody BIWA 4 Light Chain SEQ ID NO: 5

5 <400> 5

```

atggaagccc cagctcagct tctcttcctc ctgctgctct ggctcccaga taccaccgga 60
gaaattgttc tcacccagtc tccagcaacc ctgtctctgt ctccaggga gagggccacc 120
ctgtcctgca gtgccagctc aagtataaat tacatataact ggtaccagca gaagccagga 180
caggctccta gactcttgat ttatctcaca tccaacctgg cttctggagt ccctgcgcg 240
10 ttcagtggca gtgggtctgg aaccgacttc actctcacia tcagcagcct ggagcctgaa 300
gattttgccg tttattactg cctgcagtgg agtagtaacc cgctcacatt cgggtggtggg 360
accaaggtgg agattaaacg tacgggtggct gcaccatctg tcttcatctt cccgccatct 420
gatgagcagt tgaaatctgg aactgcctct gttgtgtgcc tgctgaataa cttctatccc 480
agagaggcca aagtacagtg gaaggtggat aacgccctcc aatcgggtaa ctcccaggag 540
15 agtgtcacag agcaggacag caaggacagc acctacagcc tcagcagcac cctgacgctg 600
agcaaagcag actacgagaa acacaaagtc tacgcctgcg aagtcaccca tcagggcctg 660
agctcgcccg tcacaaagag cttcaacagg ggagagtgtt ga 702

```

20 <210> 6

<211> 444

<212> PRT

<213> Artificial Sequence..

25 <220>

<223> Description of Artificial Sequence: Humanised
Murine Antibody BIWA 4 Heavy Chain SEQ ID NO: 6

<400> 6

```

30 Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
    1          5          10          15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
    20          25          30
35 Asp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
    35          40          45
Ser Thr Ile Ser Ser Gly Gly Ser Tyr Thr Tyr Tyr Leu Asp Ser Ile
40    50          55          60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
    65          70          75          80
45 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
    85          90          95
Ala Arg Gln Gly Leu Asp Tyr Trp Gly Arg Gly Thr Leu Val Thr Val
    100          105          110
50 Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser
    115          120          125
Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys
55    130          135          140
Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu
145          150          155          160

```

	Thr	Ser	Gly	Val	His	Thr	Phe	Pro	Ala	Val	Leu	Gln	Ser	Ser	Gly	Leu	
					165					170					175		
5	Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val	Pro	Ser	Ser	Ser	Leu	Gly	Thr	
				180					185					190			
	Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn	His	Lys	Pro	Ser	Asn	Thr	Lys	Val	
			195					200					205				
10	Asp	Lys	Lys	Val	Glu	Pro	Lys	Ser	Cys	Asp	Lys	Thr	His	Thr	Cys	Pro	
		210					215					220					
	Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro	Ser	Val	Phe	Leu	Phe	
15		225				230					235					240	
	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	
					245					250					255		
20	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	Phe	
				260					265					270			
	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	Pro	
			275					280					285				
25	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	
		290					295					300					
	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val	
30		305				310					315					320	
	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala	
					325					330					335		
35	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	Arg	
				340					345					350			
	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly	
			355					360					365				
40	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro	
		370					375					380					
	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser	
45		385				390					395					400	
	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln	Gln	
					405					410					415		
50	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu	His	Asn	His	
				420					425					430			
	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly	Lys					
			435					440									

55

<210> 7
 <211> 1392

<212> DNA

<213> Artificial Sequence

<220>

5 <223> Description of Artificial Sequence: Humanised
Murine Antibody BIWA 4 Heavy Chain SEQ ID NO: 7

<400> 7

```

10 atggagtttg ggctgagctg gcttttttctt gtggctatatt taaaagggtgt ccagtgtgaa 60
   gtgcagctgg tggagtctgg gggaggctta gtgaagcctg gaggggccct aagactctcc 120
   tgtgcagcct ctggattcac tttcagtagc tatgacatgt cttggggttcg ccagggtccg 180
   gggaaggggc tggagtgggt ctcaaccatt agtagtggtg gtagttacac ctactatcta 240
   gacagtataa agggccgatt caccatctcc agagacaatg ccaagaactc cctgtacctg 300
   caaatgaaca gtctgagggc tgaggacacg gccgtgtatt actgtgcaag acagggggtg 360
15 gactactggg gtcgaggaac cttagtcacc gtctcctcag ctagcaccaa gggcccatcg 420
   gtcttcccc tggcaccctc ctccaagagc acctctgggg gcacagcggc cctgggctgc 480
   ctggtcaagg actacttccc cgaaccggtg acggtgtcgt ggaactcagg cgccctgacc 540
   agcggcgtgc acaccttccc ggctgtccta cagtcctcag gactctactc cctcagcagc 600
   gtggtgaccg tgccctccag cagcttgggc acccagacct acatctgcaa cgtgaatcac 660
20 aagcccagca acaccaagggt ggacaagaaa gttgagccca aatcttgtga caaaactcac 720
   acatgcccac cgtgcccagc acctgaactc ctgggggggac cgtcagtcct cctcttcccc 780
   ccaaaacca aggacaccct catgatctcc cggacccttg aggtcacatg cgtgggtggtg 840
   gacgtgagcc acgaagaccc tgagggtcaag ttcaactggg acgtggacgg cgtggagggtg 900
   cataatgcca agacaaagcc gcgggaggag cagtacaaca gcacgtaccg tgtggtcagc 960
25 gtcctcaccg tcttgacca ggactggctg aatggcaagg agtacaagtg caaggctctcc 1020
   aacaaagccc tcccagcccc catcgagaaa accatctcca aagccaaagg gcagccccga 1080
   gaaccacagg tgtacaccct gcccccatcc cgggatgagc tgaccaagaa ccagggtcagc 1140
   ctgacctgcc tgggtcaaagg cttctatccc agcgacatcg ccgtggagtg ggagagcaat 1200
   gggcagccgg agaacaacta caagaccacg cctcccgtgc tggactccga cggctccttc 1260
30 ttcctctaca gcaagctcac cgtggacaag agcagggtggc agcaggggaa cgtcttctca 1320
   tgctccgtga tgcattgaggc tctgcacaac cactacacgc agaagagcct ctccctgtct 1380
   ccgggtaaat ga 1392

```

35

<210> 8

<211> 213

<212> PRT

<213> Artificial Sequence

40

<220>

<223> Description of Artificial Sequence: Humanised
Murine Antibody BIWA 8 Light Chain SEQ ID NO: 8

45 <400> 8

```

Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
  1                               5                               10                               15

```

50

```

Glu Arg Ala Thr Leu Ser Cys Ser Ala Ser Ser Ser Ile Asn Tyr Ile
          20                               25                               30

```

```

Tyr Trp Leu Gln Gln Lys Pro Gly Gln Ala Pro Arg Ile Leu Ile Tyr
          35                               40                               45

```

55

```

Leu Thr Ser Asn Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser
          50                               55                               60

```

```

Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro Glu

```

65

70

75

80

Asp Phe Ala Val Tyr Tyr Cys Leu Gln Trp Ser Ser Asn Pro Leu Thr
85 90 95

5

Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro
100 105 110

10

Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr
115 120 125

Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys
130 135 140

15

Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu
145 150 155 160

Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser
165 170 175

20

Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala
180 185 190

25

Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe
195 200 205

Asn Arg Gly Glu Cys
210

30

<210> 9

<211> 702

<212> DNA

<213> Artificial Sequence

35

<220>

<223> Description of Artificial Sequence: Humanised
Murine Antibody BIWA 8 Light Chain SEQ ID NO: 9

40

<400> 9

atggaagccc cagctcagct tctcttcctc ctgctgctct ggctcccaga taccaccgga 60
gaaattgttc tcaccagtc tccagcaacc ctgtctctgt ctccagggga gagggccacc 120
ctgtcctgca gtgccagctc aagtataaat tacatatact ggctccagca gaagccagga 180
caggctccta gaatcttgat ttatctcaca tccaacctgg cttctggagt ccctgcgcgc 240
45 ttcagtggca gtgggtctgg aaccgacttc actctcacia tcagcagcct ggagcctgaa 300
gattttgccg tttattactg cctgcagtgg agtagtaacc cgctcacatt cggtggtggg 360
accaaggtgg agattaaacg tacggtggct gcaccatctg tcttcatctt cccgccatct 420
gatgagcagt tgaaatctgg aactgcctct gttgtgtgcc tgctgaataa cttctatccc 480
agagaggcca aagtacagtg gaaggtggat aacgccctcc aatcgggtaa ctcccaggag 540
50 agtgtcacag agcaggacag caaggacagc acctacagcc tcagcagcac cctgacgctg 600
agcaaagcag actacgagaa acacaaagtc tacgcctgcg aagtcacca tcagggcctg 660
agctcgcccg tcacaaagag cttcaacagg ggagagtgtt ga 702

1. Use of a compound of formula

A(LB)_n

5 wherein

A is an antibody molecule which is specific for CD44;

L is a linker moiety;

B is a compound which is toxic to cells; and

n is a decimal number with $n = 1$ to 10

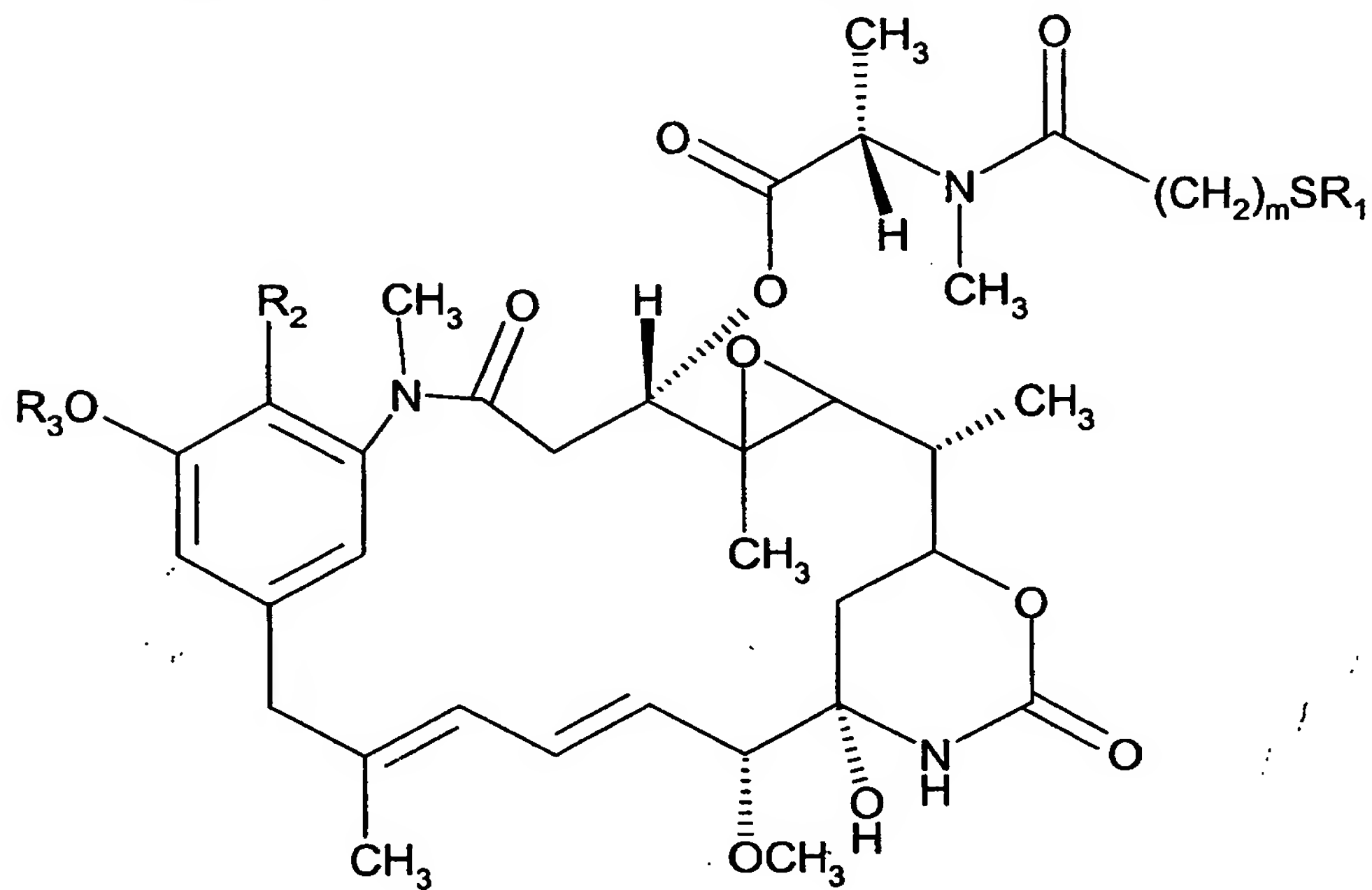
10

for the preparation of a pharmaceutical composition for the treatment of cancer,
wherein said compound is used or is for use in combination with a chemotherapeutic
agent.

- 15 2. The use of claim 1 wherein said linker moiety has a chemical bond capable of being
cleaved inside a cell.
3. The use of claim 2 wherein said chemical bond is a disulfide bond.
- 20 4. The use of claims 1 to 3, wherein the antibody molecule is specific for the exon v6 of
human CD44.
5. The use of claims 1 to 4, wherein the antibody molecule is specific for an epitope
within the amino acid sequence SEQ ID NO: 3.
- 25 6. The use of claims 1 to 5, wherein the antibody molecule is the monoclonal antibody
VFF-18 (DSM ACC2174) or a recombinant antibody having the complementary
determining regions (CDRs) of VFF-18.
- 30 7. The use of claims 1 to 6, wherein the antibody molecule comprises light chains having
the amino acid sequence SEQ ID NO: 4, or SEQ ID NO: 8, and heavy chains having
the amino acid sequence SEQ ID NO: 6.

8. The use of claims 1 to 7, wherein the toxic compound **B** is a maytansinoid.

9. The use of claim 8 wherein the maytansinoid has the formula



(Formula II)

5

wherein

R_1 represents H or SR_4 , wherein R_4 represents methyl, ethyl, linear alkyl, branched alkyl, cyclic alkyl, simple or substituted aryl, or heterocyclic;

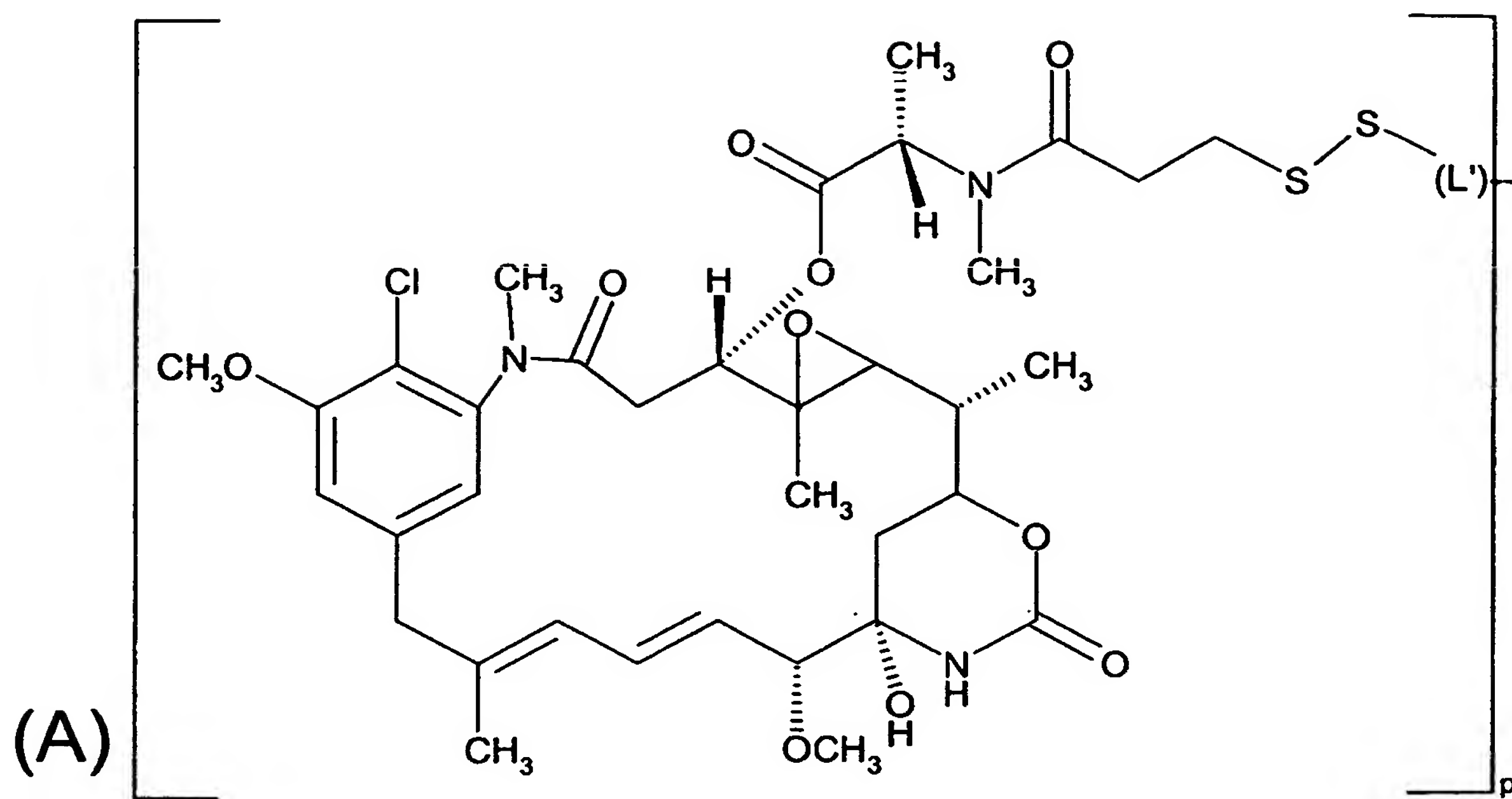
R_2 represents Cl or H;

10 R_3 represents H or CH_3 ; and

m represents 1, 2, or 3.

10. The use of claim 9, wherein R_1 is H or CH_3 , R_2 is Cl, R_3 is CH_3 , and $m = 2$

15 11. The use of claims 1 to 10, wherein the compound $A(LB)_n$ is of formula



wherein

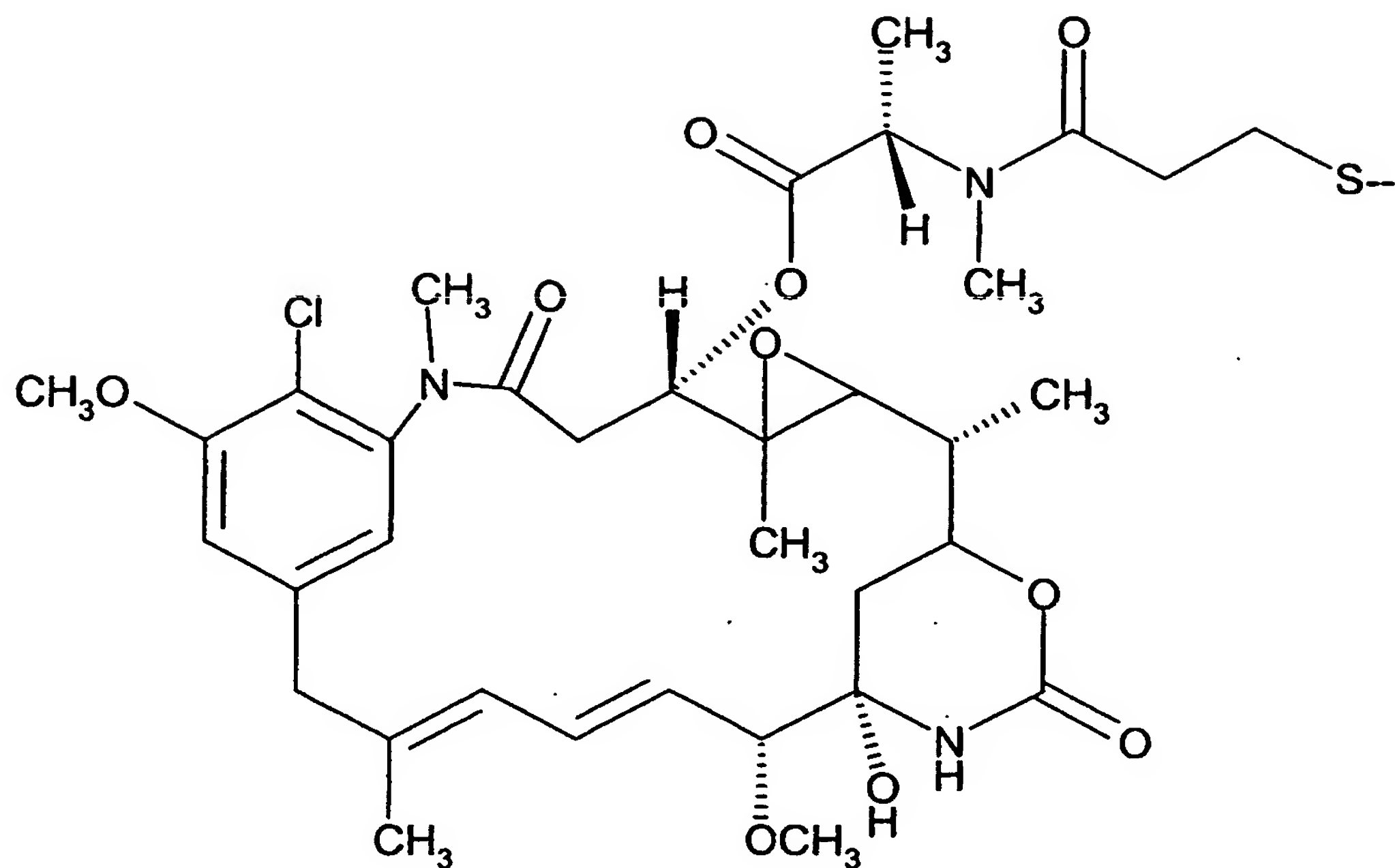
A is an antibody molecule which is specific for CD44,

(L') is an optional linker moiety

p is a decimal number with $p = 1$ to $10n$

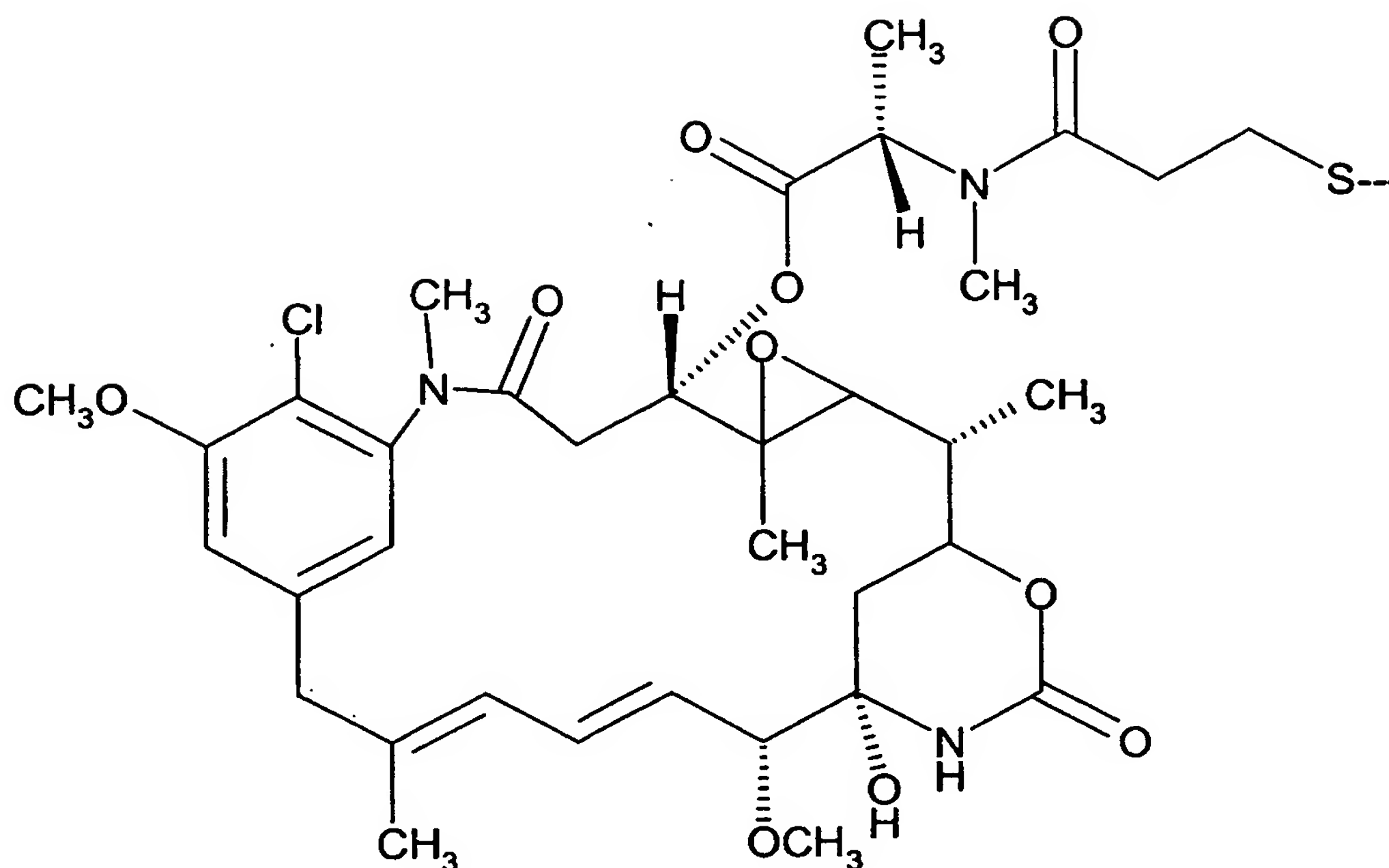
12. The use of claims 1 to 11 wherein p is 3 to 4.
13. The use of any one of claims 1 to 12, wherein the chemotherapeutic agent is a tubulin
5 binding agent.
14. The use of claims 13, wherein the chemotherapeutic agent is a microtubule stabilizing agent.
- 10 15. The use of claim 14, wherein the chemotherapeutic agent is a taxane or an epothilone.
16. The use of claim 15, wherein the chemotherapeutic agent is paclitaxel, docetaxel, RPR-116258A, epothilone A, B, C, D, E, or F, BMS-247550, or BMS-310705.

17. The use of claim 13, wherein the chemotherapeutic agent is a microtubule destabilizing agent.
18. The use of claim 17, wherein the chemotherapeutic agent is a vinca alkaloid.
- 5 19. The use of claim 18, wherein the chemotherapeutic agent is vinblastine, vincristine, vinflunine, vindesine, navelbine, or vinorelbine.
- 10 20. Use of a conjugate of a CD44v6 specific antibody molecule and a maytansinoid for the manufacture of a pharmaceutical composition for the treatment of cancer, wherein said conjugate is used or is for use in combination with a chemotherapeutic agent.
- 15 21. The use of of claim 20, wherein the antibody molecule is specific for an epitope within the amino acid sequence SEQ ID NO: 3.
22. The use of claim 21, wherein the antibody molecule is the monoclonal antibody VFF-18 (DSM ACC2174) or a recombinant antibody having the complementary determining regions (CDRs) of VFF-18.
- 20 23. The use of of any one of claims 20 to 22, wherein said antibody molecule comprises light chains having the amino acid sequence SEQ ID NO: 4, or SEQ ID NO: 8, and heavy chains having the amino acid sequence SEQ ID NO: 6.
- 25 24. The use of any one of claims 2013 to 2315, wherein the maytansinoid is linked to the antibody molecule by a disulfide moiety.
25. The use of any one of claims 20 to 24, wherein the maytansinoid has the formula



Formula (IV)

26. The use of a conjugate of a CD44v6 specific antibody molecule and a maytansinoid for the manufacture of a medicament for the treatment of cancer, wherein said compound is used or is for use in combination with a chemotherapeutic agent, and wherein the antibody comprises light chains having the amino acid sequence SEQ ID NO: 4, and heavy chains having the amino acid sequence SEQ ID NO: 6, and wherein the maytansinoid has the formula



(Formula IV)

and is linked to the antibody through a disulfide bond.

27. The use of any one of claims 20 to 26, wherein one or more maytansinoid residues are linked to an antibody molecule.

28. The use of claim 27, wherein 3 to 4 maytansinoid residues are linked to an antibody molecule.

29. The use of any one of claims 20 to 28, wherein the maytansinoid is linked to the antibody molecule through a $-S-CH_2CH_2-CO-$, a $-S-CH_2CH_2CH_2CH_2-CO-$, or a $-S-CH(CH_3)CH_2CH_2-CO-$ group.

30. The use of any one of claims 20 to 29, wherein the chemotherapeutic agent is a tubulin binding agent.

31. The use of claims 30, wherein the chemotherapeutic agent is a microtubule stabilizing agent.

32. The use of claim 31, wherein the chemotherapeutic agent is a taxane or an epothilone.

33. The use of claim 32, wherein the chemotherapeutic agent is paclitaxel, docetaxel, RPR-116258A, BMS-247550, BMS-310705, or epothilone A, B, C, D, E, or F.
- 5 34. The use of claim 30, wherein the chemotherapeutic agent is a microtubule destabilizing agent.
35. The use of claim 34, wherein the chemotherapeutic agent is a vinca alkaloid.
- 10 36. The use of claim 35, wherein the chemotherapeutic agent is vinblastine, vincristine, vindesine, vinflunine, navelbine, or vinorelbine.
37. The use of any one of claims 1 to 36, wherein the cancer is head and neck squameous cell carcinoma, esophagus squameous cell carcinoma, lung squameous cell carcinoma, skin squameous cell carcinoma, cervix squameous cell carcinoma, breast
15 adenocarcinoma, lung adenocarcinoma, pancreas adenocarcinoma, colon adenocarcinoma, or stomach adenocarcinoma.
38. The use of any one of claims 1 to 37, wherein said compound $A(LB)_n$ or conjugate, and
20 said chemotherapeutic agent are formulated in separate pharmaceutical compositions.
39. The use of any one of claims 1 to 37, wherein said compound $A(LB)_n$ or conjugate and said chemotherapeutic agent are formulated in one single pharmaceutical composition.
- 25 40. Method of treatment of cancer in a patient in need thereof, comprising administering to the patient a therapeutically effective amount of a compound $A(LB)_n$ as defined in any one of claims 1 to 12, or a conjugate as defined in any one of claims 20 to 29, in combination with a chemotherapeutic agent as defined in any one of claims 13 to 19, or 30 to 36.
- 30 41. The method of claim 40, wherein the cancer is head and neck squameous cell carcinoma, esophagus squameous cell carcinoma, lung squameous cell carcinoma, skin squameous cell carcinoma, cervix squameous cell carcinoma, breast adenocarcinoma,

lung adenocarcinoma, pancreas adenocarcinoma, colon adenocarcinoma, or stomach adenocarcinoma.

42. The method of claim 40 or 41, wherein the compound $A(LB)_n$ or conjugate, and the
5 chemotherapeutic agent are administered separately.

43. The method of claim 40 or 41, wherein the compound $A(LB)_n$ or conjugate, and the
 chemotherapeutic agent are administered as components of a single pharmaceutical
 composition.

10

44. Pharmaceutical composition comprising a compound $A(LB)_n$ as defined in any one of
 claims or according to claims 1 to 12, or a conjugate as defined in any one of claims 20
 to 29, together with a chemotherapeutic agent as defined in any one of claims 13 to 19,
 or 30 to 36, and optionally further comprising one or more pharmaceutically acceptable
15 carrier(s), diluent(s), or excipient(s).

20

45. A kit comprising, in separate pharmaceutical compositions, a compound $A(LB)_n$ as
 defined in any one of claims 1 to 12, or a conjugate as defined in any one of claims 20
 to 29, and a chemotherapeutic agent as defined in any one of claims 13 to 19, or 30 to
 36.

25

46. Use of a chemotherapeutic agent for the preparation of a pharmaceutical composition
 for the treatment of cancer, wherein said chemotherapeutic agent is used or is for use in
 combination with a compound of Formula $A(LB)_n$ as defined in any of the preceding
 claims.

30

47. Use of a chemotherapeutic agent for the preparation of a pharmaceutical composition
 for the treatment of cancer, wherein said chemotherapeutic agent is used or is for use in
 combination with a conjugate as defined in any one of claims 20 to 29.

48. The use of claim 46 or 47, wherein the chemotherapeutic agent is a taxane, an
 epothilone, a vinca alkaloid, a platinum compound, a camptothecin, a cryptophycin, a
 dolastatin, a 5,6-dihydroindolo[2,1-a]isoquinoline derivative, a spongistatin, an

epipodophyllotoxin, an alkylating agent, an purine antagonist, a pyrimidine antagonist, or a DNA intercalator.

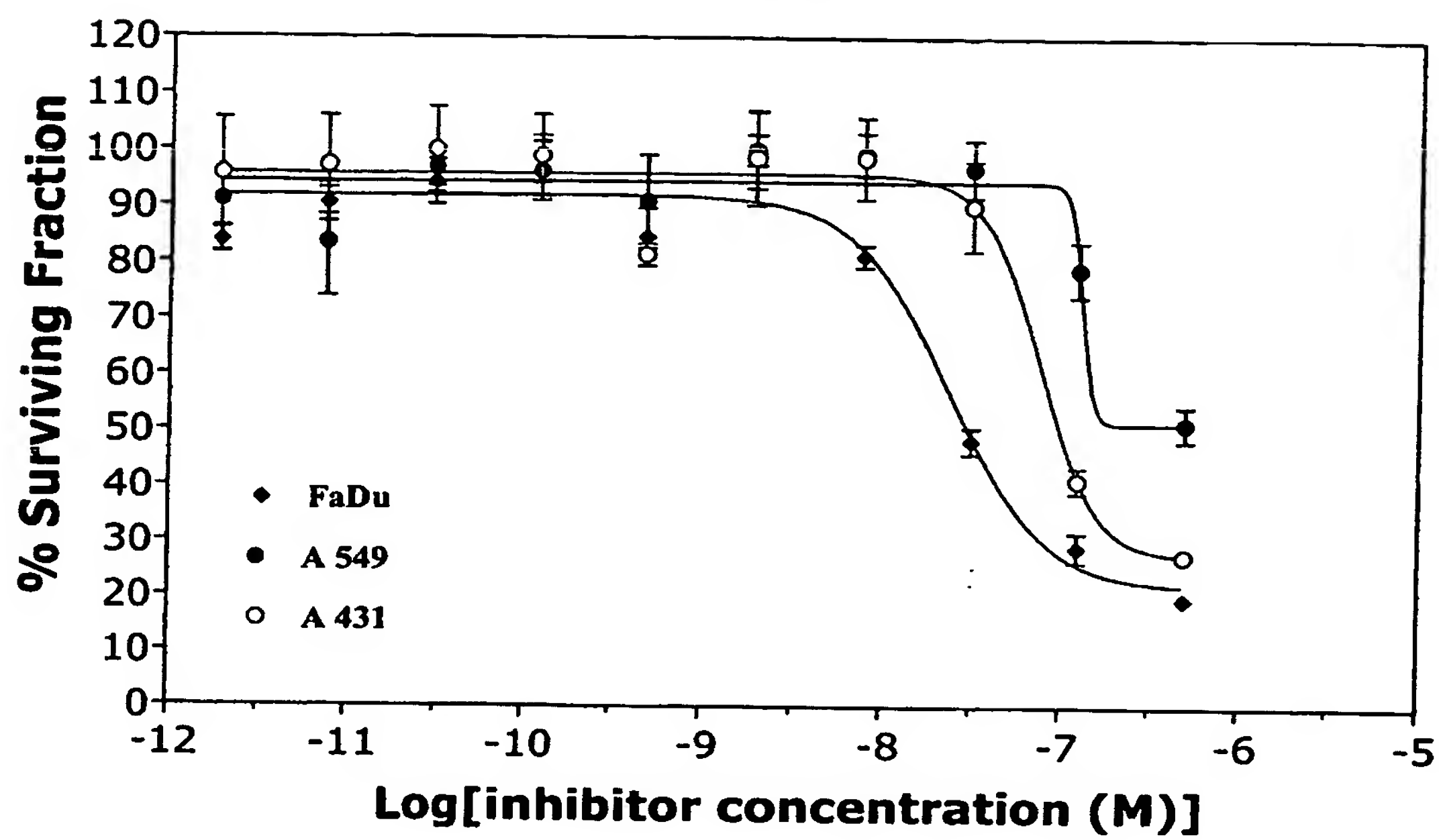
49. The use of claim 48, wherein the chemotherapeutic agent is docetaxel, paclitaxel, RPR-
5 116258A, epothilone A, B, C, D, E, or F, BMS-247550, BMS-310705, vinblastine, vindesine, vincristine, vinorelbine, vinflunine, navelbine, combretastatin A4-phosphate, hydroxphenastatin, AVE 8062, spongistatin 1, 2, 3, 4, 5, 6, 7, 8, or 9, E-7010, dolastatin, cemadotin hydrochloride, mivobulin isethionate, cryptophycin, camptothecin, topotecan, irinotecan, 9-aminocamptothecin, cisplatin, carboplatin,
10 oxaliplatin, iproplatin, ormaplatin, tetraplatin, etoposide, teniposide, doxorubicin, daunorubicin, dactinomycin, plicamycin, mitomycin, bleomycin, idarubicin, cyclophosphamide, mechlorethamine, melphalan, chlorambucil, procarbazine, dacarbazine, altretamine, carmustine, lomustine, semustine, methotrexate, mercaptopurine, thioguanine, fludarabine phosphate, cladribine, pentostatin,
15 fluorouracil, cytarabine, or azacytidine.

Abstract

**EPO - Munich
80
21. Aug. 2002**

The invention relates to the combined use of conjugates of CD44 specific antibodies with cytotoxic compounds and chemotherapeutic agents in cancer therapy, pharmaceutical compositions comprising such compounds and/or chemotherapeutic agents, and methods of cancer treatment. Preferred conjugates contain maytansinoids as cytotoxic compounds, and preferred chemotherapeutic agents are taxanes, epothilones, and vinca alkaloids.

21. Aug. 2001

BIWI 1**Figure 1**

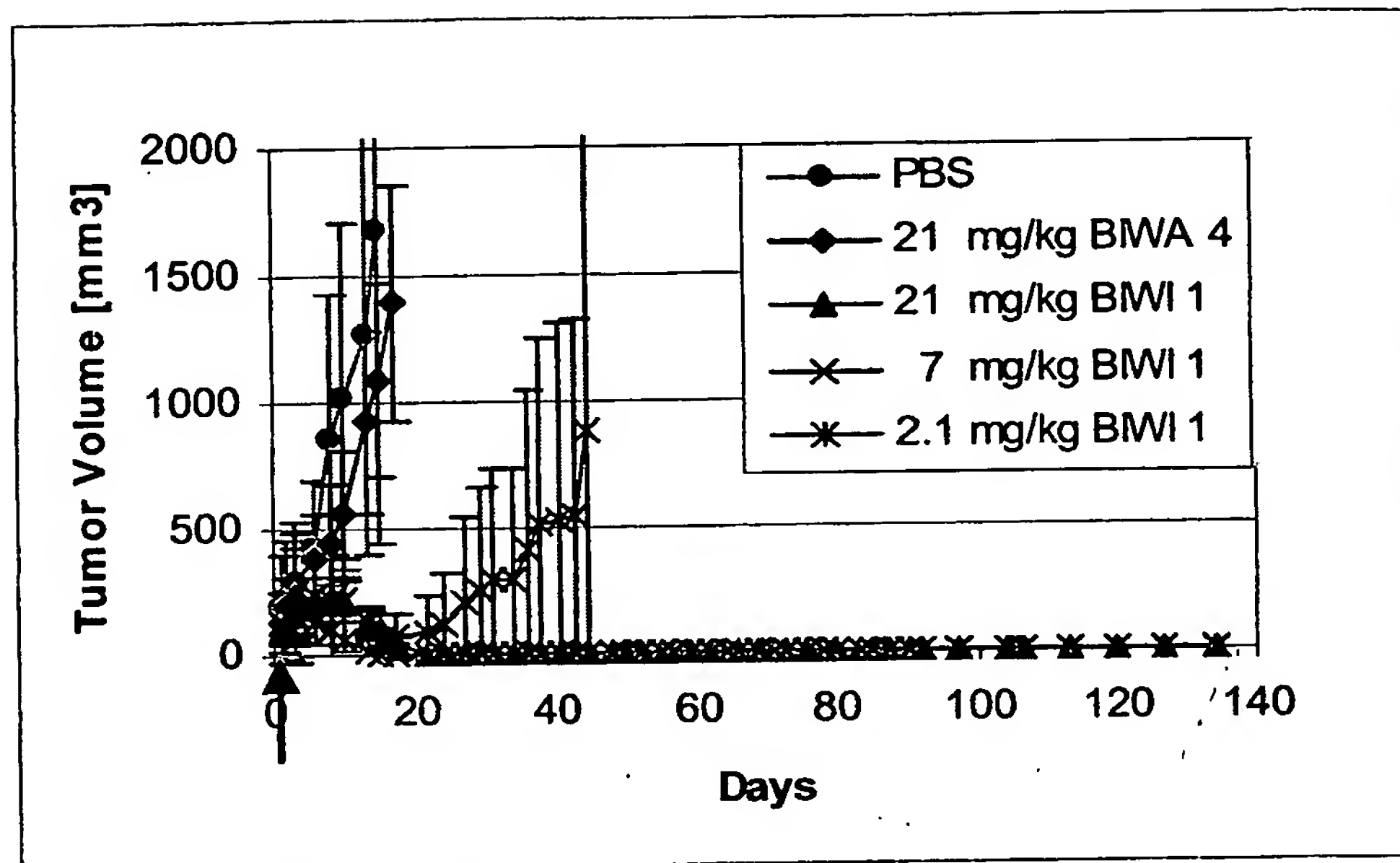


Figure 2

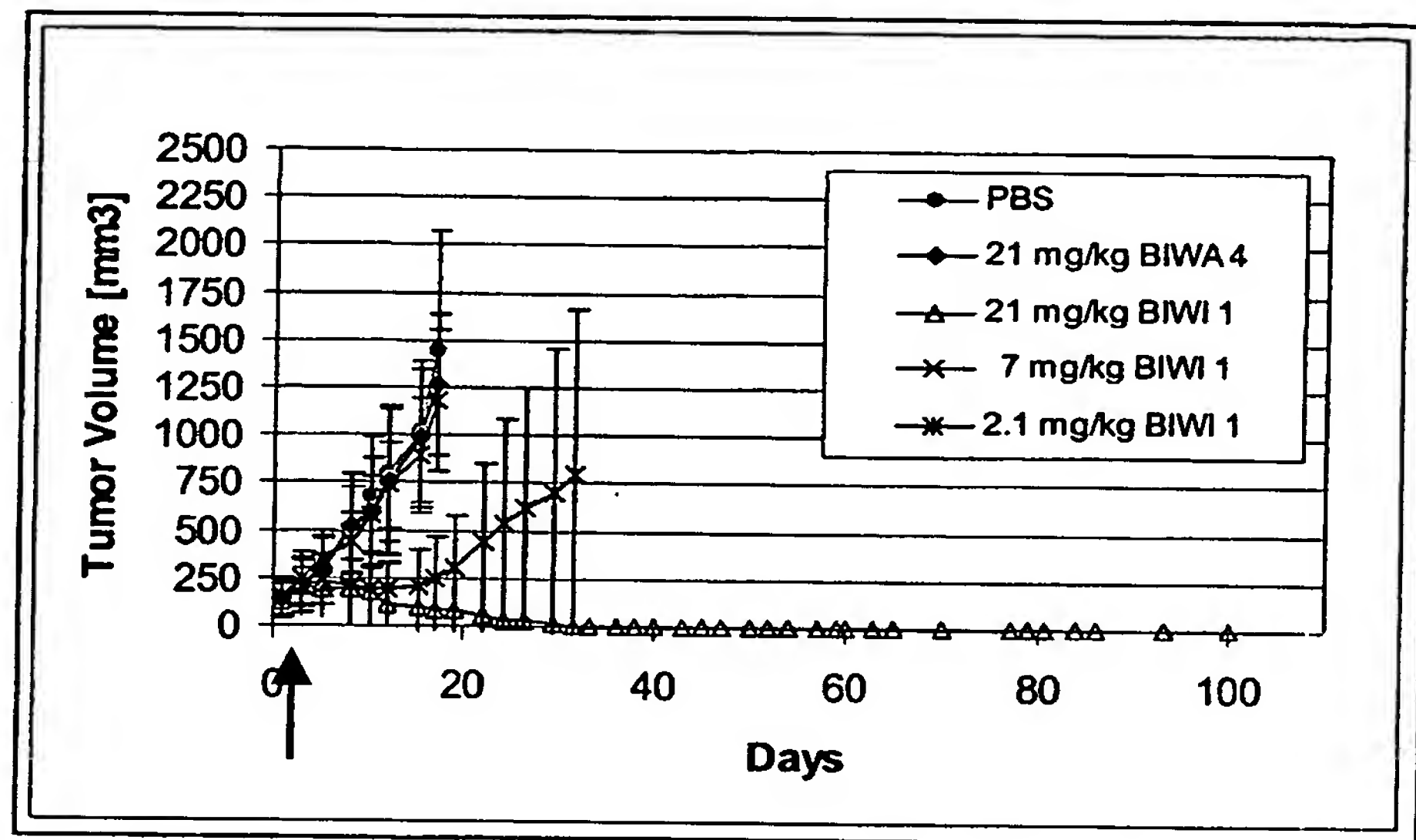
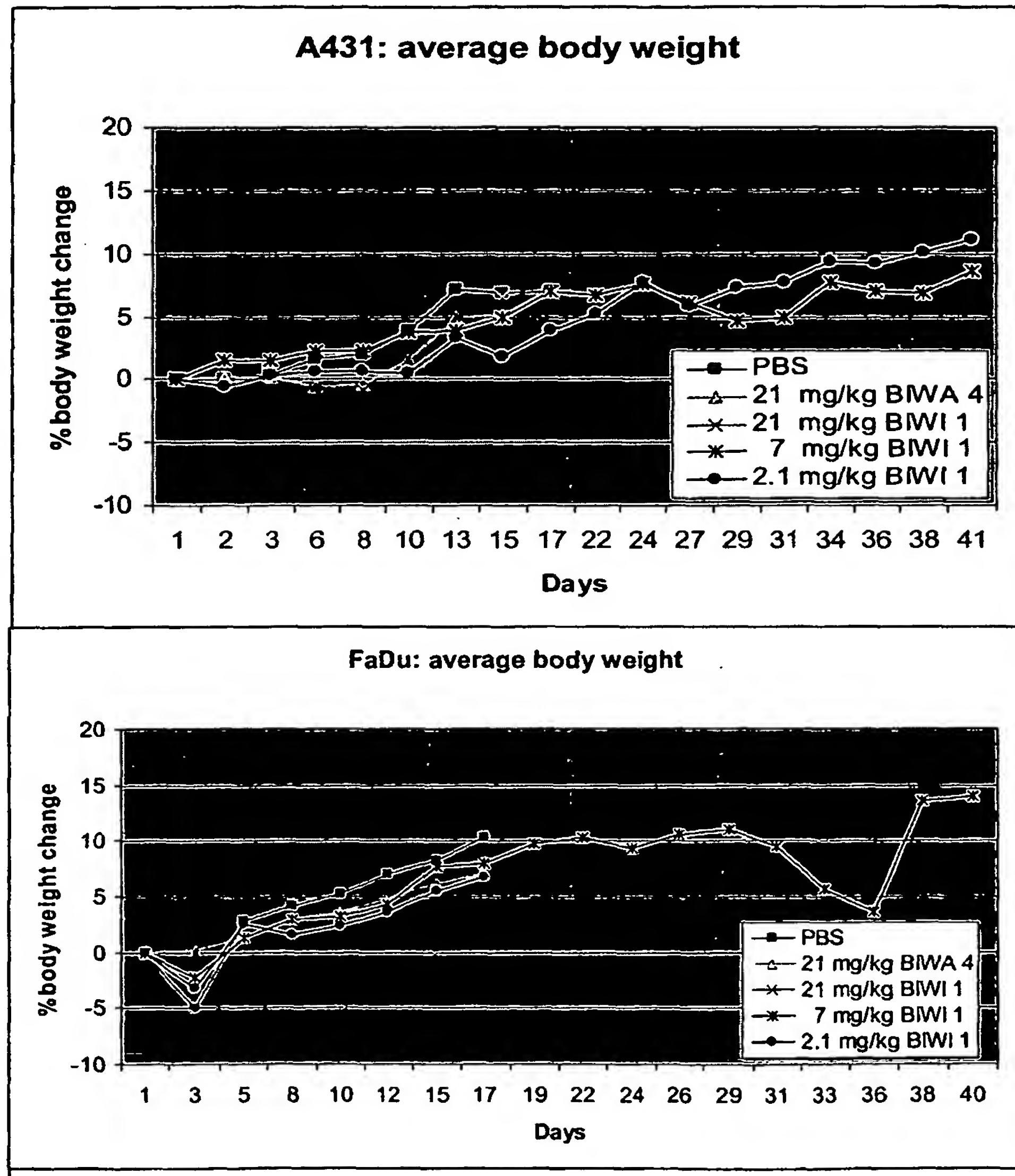
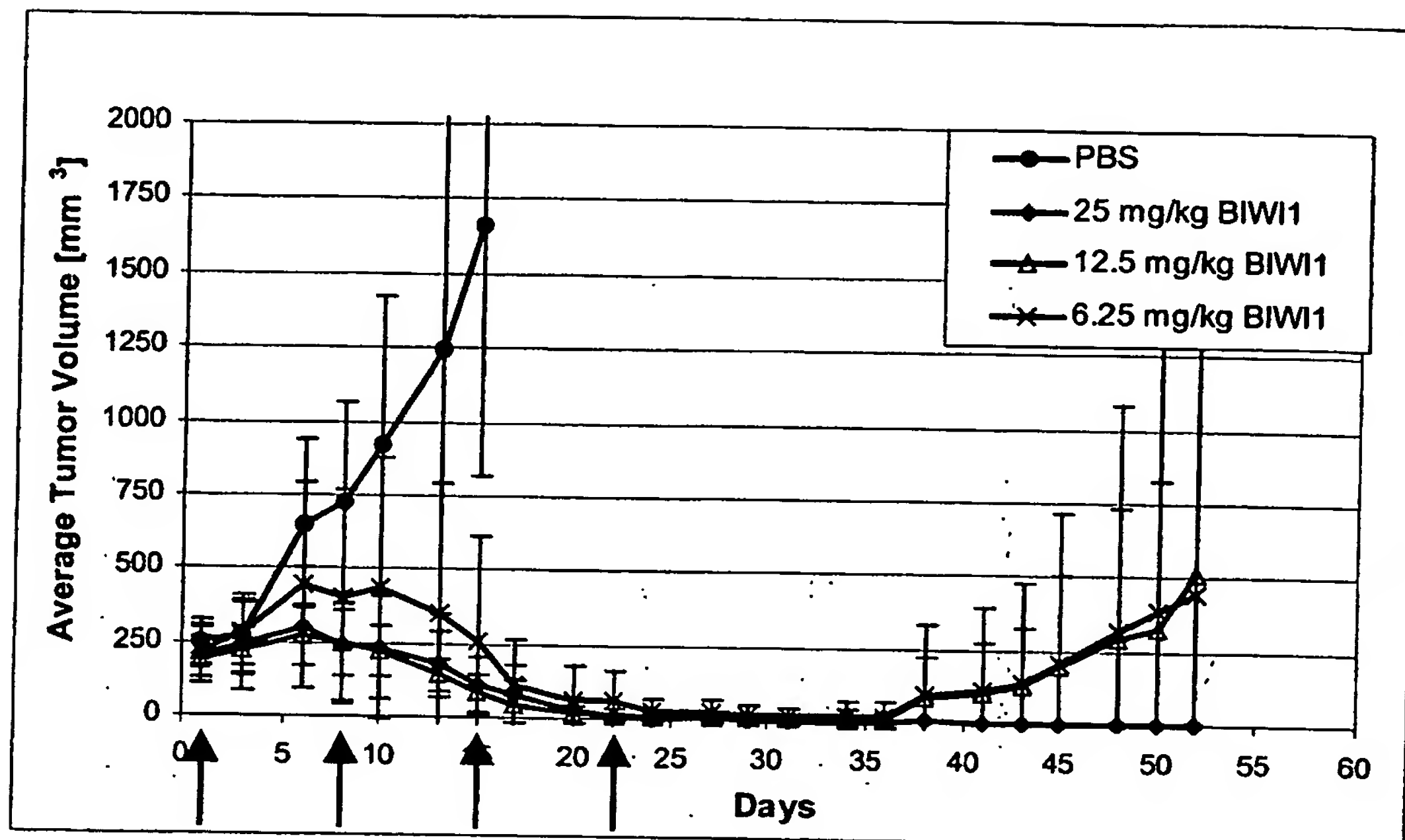
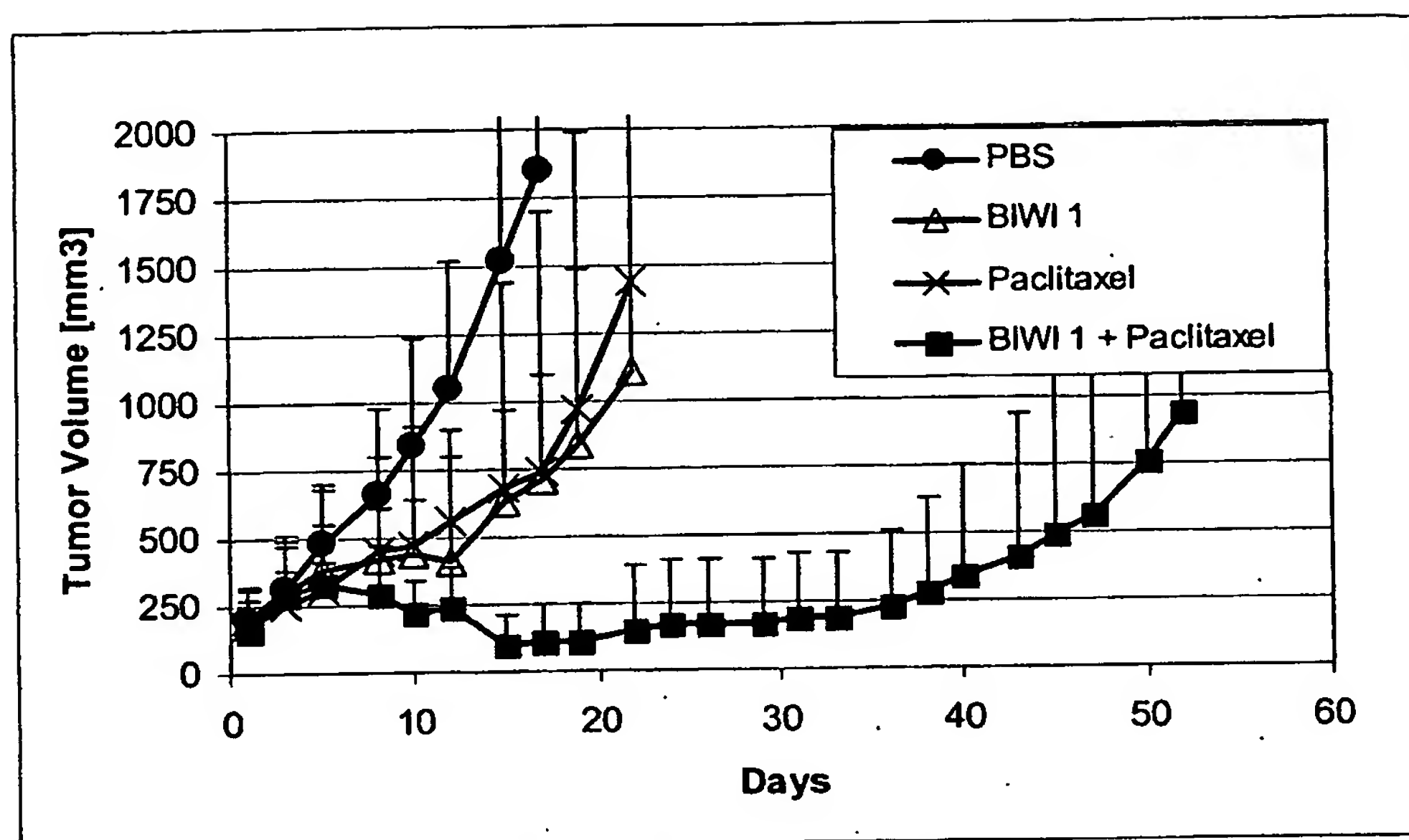
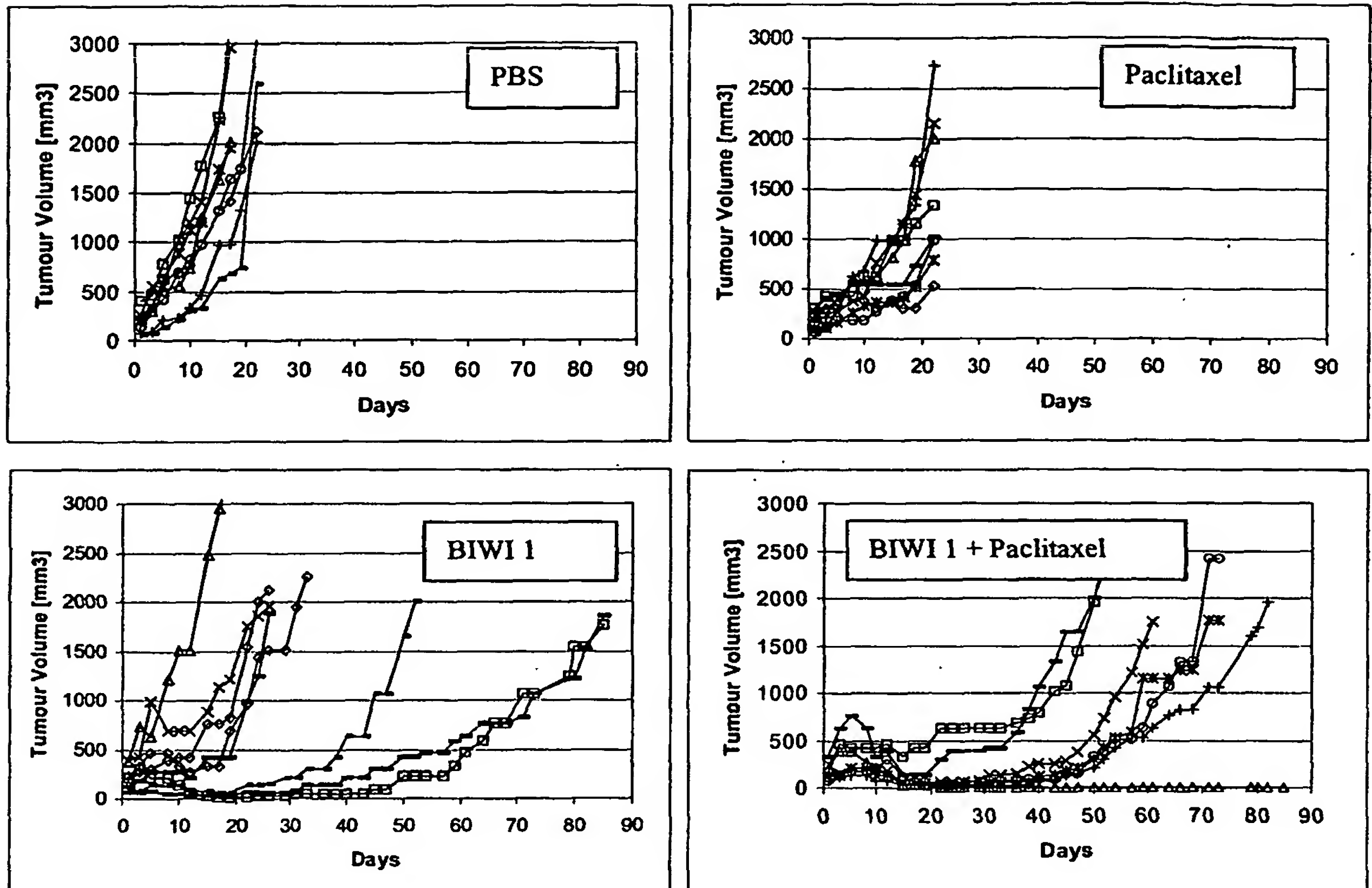


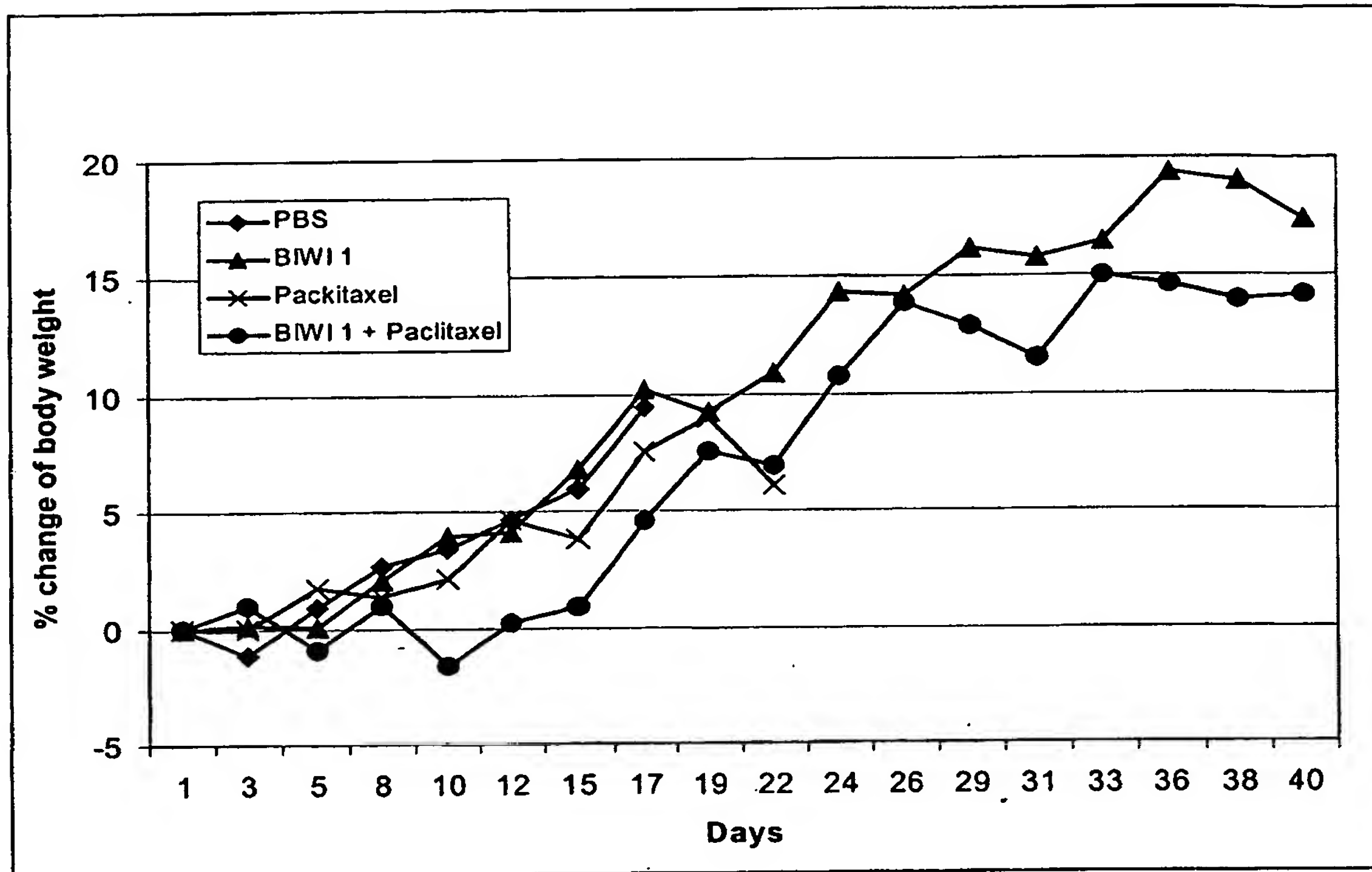
Figure 3

**Figure 4**

**Figure 5**

**Figure 6**

**Figure 7**

**Figure 8**